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THE VENOM OF HELODERMA

BY

LEO LOEB

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WITH THE COLLABORATION OF CARL L. ALSBERG, ELIZABETH COOKE, ELLEN P. CORSON-WHITE, MOYER S. FLEISHER, HENRY FOX, T. S. GITHENS, SAMUEL LEOPOLD, M. K. MEYERS, M. E. REHFUSS, D. RIVAS, AND LUCIUS TUTTLE

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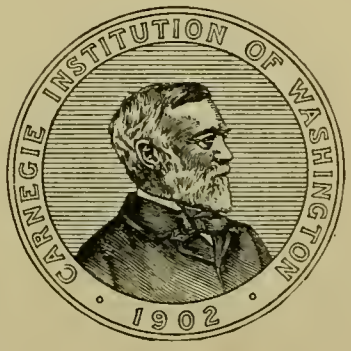

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PREFACE.

Our investigations into the properties and toxic action of the venom of *Heloderma* were undertaken at the request of Dr. S. Weir Mitchell, and carried out with the aid of grants from the Carnegie Institution of Washington.

In order to be able to extend the researches in various directions, I associated with myself a number of collaborators, who undertook the study of various parts of the problem. If, notwithstanding this partition, the work has, as I hope, preserved its uniform character, it is due to the fact that by far the greater part of these investigations was carried out in the Laboratory of Experimental Pathology of the University of Pennsylvania, of which I had charge at that time. This made possible the constant correlation of the results, and the data obtained in the study of one problem became available for and were used in the investigation of other problems. The chemical analysis of the venom which Doctor Alsberg undertook was done elsewhere. The histological study of the changes produced by the venom in the central nervous system was partly done in the Laboratory of Neuropathology of the University of Pennsylvania, through permission of Dr. W. G. Spiller.

Dr. D. T. MacDougal, Director of the Department of Botanical Research of the Carnegie Institution of Washington, on various occasions kindly obtained for us living specimens of *Heloderma suspectum*.

To Dr. S. Weir Mitchell and to the Smithsonian Institution we are indebted for a specimen of *Heloderma horridum*.

I am under obligation to Doctor Calmette, of the Pasteur Institute of Lille, who very kindly put at my disposal some of his cobra antivenin.

I wish to thank all my collaborators for their faithful cooperation, and especially to acknowledge the assistance given me in the preparation of this work on various occasions by Dr. Moyer S. Fleisher and Dr. Ellen P. Corson-White.

Leo Loeb.

Barnard Free Skin and Cancer Hospital,
St. Louis, Missouri, March 1912.

CONTENTS.

	PAGE.
Preface	III
Introduction. By Leo Loeb	1
I. Morphology of the Poison Gland of Heloderma	15
A. Anatomy of the Poison Gland of Heloderma. By Henry Fox	17
B. Structural Changes produced in the Poison Gland by Injection of Pilocarpine. By Henry Fox (with the collaboration of Leo Loeb)	29
C. Transplantation of the Venom Gland. By Henry Fox and Leo Loeb	35
D. Solubility of the Venom Granules. By Henry Fox and Leo Loeb	45
II. General Properties and Actions of the Venom of Heloderma, and Experiments in Immunization. By Elizabeth Cooke and Leo Loeb (with some additional experiments by Moyer S. Fleisher)	49
Review of the Literature	51
Method of Collecting the Venom and Factors which influence the Amount of Secretion	52
Influence of Pilocarpine on the Secretion of Venom	53
Effect of Heat on Venom	55
Effect of Standing in Solution on Toxicity of Venom	57
Effect of Dialyzing	58
Effect of Filtration	60
Influence of the Addition of Acid to Venom Solution	61
Effect of Roentgen Rays on Secretion of Venom Gland	61
Effects of Venom upon Animals	62
Condition of Mouse's Eye produced by Injection of Heloderma Venom	64
Anatomical Lesions produced by Injection of Venom	66
Histological Changes produced by Injection of Venom	69
Influence of the Method of Administration on the Toxicity of the Venom	71
Effect of placing Collodion Capsules containing Venom in the Peritoneal Cavity	74
Administering Fractional Doses of Venom	76
Susceptibility of Various Species of Animals to Heloderma Venom	77
Toxicity of the Organs and Excretions of Heloderma	81
Influence of Venom on Coagulation Time of the Blood	83
Influence of Inoculation of Pieces of Venom Gland into Mice	86
Immunization against Heloderma Venom	88
Protective Power of the Serum of Immunized Rabbits	90
Precipitins in the Serum of Rabbits immunized against Heloderma Venom	92
Does Blood Serum of Heloderma contain a Venom Antitoxin?	93
Does the Injection of Venom produce a State of Anaphylaxis?	94
III. Bacteriology of the Saliva of Heloderma Suspectum. By D. Rivas	97
IV. Influence of Heloderma Venom upon Blood Pressure, Diuresis, Peritoneal Transudate, and Intestinal Fluid. By Moyer S. Fleisher	101
V. Effect of the Venom of Heloderma Suspectum on the Isolated Heart. By Thomas Stotesbury Githens	119
VI. Experimental Production of Acute Gastric Ulcer in the Guinea-Pig. By M. E. Rehfuss	125

PAGE.

VII. Histological Changes of the Central Nervous System produced by Heloderma Venom. By Samuel Leopold 137
VIII. Hemolytic Properties of Heloderma Venom. By Elizabeth Cooke and Leo Loeb 143
IX. Action of Heloderma Venom on the Cellular Elements of the Blood within the Living Organism. By M. K. Meyers and Lucius Tuttle 171
X. The Influence of Heloderma Venom upon Phagocytosis. By Lucius Tuttle. 187
XI. Can the Presence of Antibodies to the Venom of Heloderma Suspectum be Demonstrated in the Blood Serum of this Animal by the Method of Complement Fixation? By Ellen P. Corson-White 193
XII. Action of Calmette's Cobra Antivenin upon the Venom of Heloderma. By Moyer S. Fleisher and Leo Loeb 199
XIII. Influence of Various Stains upon Venom. By Lucius Tuttle 205
XIV. Absorption of Heloderma Venom by Suspensions of Various Substances. By Moyer S. Fleisher and Leo Loeb 211
XV. Biochemical Studies upon the Venom of Heloderma Suspectum. By Carl L. Alsberg 229
1. The Chemical Character of Venom.
2. The Enzymes of the Venom and of the Venom Gland.

THE VENOM OF HELODERMA.

INTRODUCTION.

While considerations of a practical as well as of a theoretical nature led to the study of snake venoms, the interest in the venom of *Heloderma* is more purely scientific and of comparatively slight practical importance. No death of a human being has come to our knowledge that can be attributed to the bite of a Gila monster. A bite from this animal is in man either followed by no symptoms at all or by a local swelling, perhaps extending to the shoulder, if the bite affected the upper extremity. The swelling disappears within a short time, but it may be followed by a long-continued weakness of the hand, with sensations of discomfort lasting over several years. The preparation of an antivenin so desirable in the case of snake bites is therefore of little importance in the case of *Heloderma*. From a theoretical point of view, on the other hand, a study of the venom of *Heloderma* is of considerable interest. We have learned much of the mode of action and of the constitution of snake venoms through the extensive studies of S. Weir Mitchell and Reichert, Calmette, Lamb, Flexner and Noguchi, Keyes, Fraser, von Dungern and Coca, Bang, and especially Faust, with many others.

Relatively little of a definite character is known concerning the venom of *Heloderma*. The investigations of Santesson and van Denburgh and Wight, which followed the observations of S. Weir Mitchell and Reichert, were necessarily limited in their scope, mainly in consequence of the small amount of venom at their disposal. Data which permit a comparison between the mode of action and constitution of snake venoms and the venom of the only other poisonous reptile known, the *Heloderma*, are of great theoretical interest. An intimate understanding of the different steps by means of which the venom gains entrance into certain cells and exerts its destructive action on the constituents of these cells is of fundamental pathological importance. The number of unknown factors coming into play during these processes is, however, very great, and the number of equations with which we can operate must be proportionately increased. We undertook, therefore, to supply the data which will render more accessible the venom of *Heloderma* for future detailed study of certain problems. Not rarely our own investigations had to cease when the point was reached where more far-reaching studies promised to yield results of importance, and the further cultivation of this field had to be left to future investigations. Under these circumstances it may not be without interest to point out some of the results of our investigations, and especially those findings which may serve as a starting-point for future research.

The poison glands of *Heloderma* and of snakes differ somewhat in position and structure. The poison gland of snakes is considered to be homologous to the parotid of mammals, while in *Heloderma* it corresponds to a sublabial

gland. Their microscopic structure also differs somewhat. In *Heloderma* the intralobular ducts alone contain the typical granules and the end acini do not seem to be concerned in the production of the venom, while in snakes the acini as a whole are believed to secrete the venom. Doctor Fox has not been able to find mucus-gland cells in *Heloderma*, while in snakes they seem to occur. The mechanism of the poison gland also differs in *Heloderma* and in snakes. In the latter the contraction of a muscle-coat surrounding the gland expresses the venom, while in *Heloderma* the contraction of certain muscles makes tense a fibrous facia which then presses the venom from the gland. Differences also exist in regard to the structure of the teeth of *Heloderma* and poisonous snakes. In different though related species the same end, *i. e.*, the secretion of venom during the process of biting, is achieved through means which are in some respects similar but not identical. Different glands can be adapted to a similar purpose and variations can occur in the mechanism of the expulsion and in the chemical constitution of the venom. If we consider, furthermore, the differences in the constitution of the venom, we may conclude that the production and secretion of venom are effected through different means in various, even nearly related, species of animals.

No direct proof has so far been given that the granules are the bearer of the venom within the cell, or that the granules are concerned in the production of the venom. Our observations on the action of pilocarpine upon the venom gland suggest, however, very strongly that the granules do carry the venom or its precursor. Pilocarpine increases the secretion of the venom and causes at the same time a rapid disappearance of the granules. After repeated injections of the pilocarpine at intervals of 24 hours the new formation of the granules becomes imperfect, and pilocarpine correspondingly fails to call forth an increased flow of venom. After transplantation, the toxic character of the gland is retained and we find granules present in a certain number of lobules. If we consider the great lability of the granules, it becomes probable that these granules have been newly formed in the transplanted glands and are not merely the granules that existed before transplantation. On the whole, the gland preserves its character very well after transplantation, especially when we consider that the gland functionates under modified conditions where an expulsion of the secreted material is no longer possible.

The significance of cell-granules has been the subject of much controversy. Therefore the data which we give concerning the behavior of the granules in the poison gland may not be without interest. The granules are, on the whole, very labile; many dissolve spontaneously in the venom. Distilled water, weak acid, and extremely weak alkali cause their dissolution. Addition of NaCl solution, especially of hypertonic solutions, renders them more stable. In this respect we notice a similarity between the granules of the poison gland and of the blood-cells of *Limulus*, which latter I studied on previous occasions.[1] Both kinds of granules are labile and are preserved in a similar manner by salt solution. These and certain other observations seem to point to the conclusion

[1] Folia Hæmatologica, vol. 4, 1907; Pflüger's Archiv, vol. 131, 1910.

that the granules consist of a proteid rather than a lipoid material, although a mixture of both may be present.

After addition of slightly hypertonic salt solution, a relatively stable suspension of granules (with some admixture of nuclei) can be obtained, and through centrifugation it will perhaps be possible to separate the granules and the liquid part of the venom. A comparison of the toxicity of these two constituents of the venom could thus be made and direct proof obtained of the significance of the granules in the production or fixation of the venom.

There exists much difference of opinion in regard to the real function of the poison gland. Does it merely take up from the blood the poisonous material produced by other cells, or does it actually manufacture the venom from non-poisonous substances supplied through blood or lymph? Calmette and Faust accepted the first view. In snakes the blood and, as Flexner and Noguchi found, even the ova contain a venom very similar to that secreted by the poison gland. Calmette and Faust believe, therefore, that the cells of the poison gland have a selective affinity for the venom circulating in the blood. Inasmuch as Calmette noticed that the heat resistance of the poisonous substance present in the blood differs somewhat from that of the venom secreted by the poison gland, he assumes that a precursor which is poisonous and has been produced by other cells is slightly modified in the poison gland and then secreted. This difference in the heat resistance of the two poisons he regards as a reason sufficient to exclude the view of Phisalix and Bertrand, who regard the venom circulating in the blood as being manufactured in the venom gland and eliminated in the blood through a mechanism related to internal secretion. They found, accordingly, that after extirpation of the venom gland of snakes the blood lost its toxic properties.

In *Heloderma* conditions are less complicated. Here the venom can not be found anywhere in the body except in the poison gland. Neither does the blood become poisonous after extirpation of the poison glands, as should be expected if the poison glands served merely as a place of elimination for the venom prepared elsewhere. We can therefore be certain that in the case of *Heloderma* the poison gland is the place where the venom is produced out of non-poisonous material carried to the gland. Such a conclusion is also in accordance with the view that the granules of the gland are the carriers of the venom.

In its action on animals the venom of *Heloderma* bears a close resemblance to the venoms of some snakes, especially the Colubridæ. It affects mainly the central nervous system. The marked local, especially the hemorrhagic, effects that characterize the venom of some vipers are absent. It affects very early the respiratory center and, as in the case of cobra venom, death is mainly due to a failure in the action of this center. Accordingly, we may find, even within the first hour after injection of the venom, microscopic changes in the ganglia-cells. The changes here are therefore noticeable at least as early as in the case of snake venoms that have been investigated from this point of view; but the changes do not go so far as after injection of the venom of some Colu-

bridæ studied by Hunter and Lamb. In contradistinction to these venoms, the venom of *Heloderma*, although principally a neurotoxin, does not entirely destroy the ganglia-cells. Its effect on the heart is very slight *in vitro* as well as *in vivo*. Furthermore, it is of interest that the action of the venom on the heart is reversible and that removing the venom can restore the heart action (Githens).

We also observe, in contradistinction to certain snake venoms with the power to dissolve muscle-tissue, that the venom of *Heloderma* does not exert a lytic effect upon the heart-muscle. The marked primary fall in blood-pressure which takes place after injection of the venom must therefore be due to a direct or indirect vasomotor action of the venom on the blood-vessels. In this respect our results agree with those obtained by van Denburgh and Wight. Fleisher, however, did not observe the strong antagonistic action of adrenalin upon the venom noticed by the previous investigators. Furthermore, he could establish the fact that the diminution in the flow of urine during a long-continued injection of venom dissolved in much 0.85 per cent NaCl solution is not due to a direct injurious action upon the kidney on the part of the venom, but corresponds to, and is merely the result of, the decrease in blood-pressure. The interchange of fluid between the blood and body cavities is not markedly influenced by the venom during an infusion with 0.85 per cent NaCl solution. The elimination of fluid into the small intestines is, however, increased under these conditions, and correspondingly we find that if death follows an injection of venom, the small intestine contains much fluid; especially is this true of the guinea-pig.

Two sequels of poisoning with the venom of Gila monster deserve special mention, inasmuch as I have found no reference to them in the literature on snake venoms, so far as the latter was accessible to me: (1) In the mouse we find very frequently a rupture of blood-vessels near the optic nerve and protrusion of the eyeball with subsequent opacity of the cornea or lens. (2) In guinea-pigs we found ulcers and hemorrhages in the wall of the stomach. The investigations of M. E. Rehfuss have shown that these ulcers are due to a digestive action of the gastric juice; that the hemorrhages are usually secondary; that this effect is not specific for venom, but is found after the administration of a great variety of poisonous substances in cases in which the animal is markedly affected by the toxic substance. Thrombi are present in the neighborhood of the lesions, but are not the cause of the lesions. The factor that makes the mucosa accessible to the digestive action of the gastric juice has yet to be determined. Comparative studies in different species of animals (herbivorous and carnivorous animals) ought to yield results of interest.

Other structural changes produced by the venom are slight and seem to be especially noticeable after chronic poisoning. Even the slow action after introduction into the peritoneal cavity of collodion capsules containing venom caused no marked structural changes; they certainly formed a much less prominent feature than the great loss of weight which we observed in such animals. A study of metabolism under these conditions might be of interest and throw light on the disturbances of metabolic equilibrium in states of chronic poisoning.

On the whole, we may conclude that the structural changes (even in the brain) which we find after the administration of venom are not the cause of the primary pathologic effects, but either accompany or follow the latter. Later, however, these functional disturbances may give rise to secondary structural changes, a consideration which probably applies also to the changes which Leopold found in the ganglia-cells of animals injected with poison of Gila monster.

The effect of the venom of *Heloderma* upon the cellular elements, as well as upon the coagulation of the blood, is less marked than those found by previous investigators in the case of the venoms of various snakes. If there exists any effect at all upon the coagulation of the blood, it is certainly very slight. After intravenous injections of extracts of the poison gland of *Heloderma*, we observed an insignificant retardation in the coagulation of the blood, an effect frequently observed after intravenous injection of various tissue extracts in a quantity too small to cause intravascular clotting.

The venom of *Heloderma* possesses no direct hemolytic action. It becomes hemolytic only in combination with some activators, especially lecithin and certain blood sera, and even here the dose of lecithin necessary for activation is relatively great. In the case of heloderma, as well as cobra venom, the hemolytic is somewhat less heat-resistant than the neurotoxic substance, while the neurotoxin of the venom of *Heloderma* is somewhat more heat-resistant than that of cobra venom; the hemolysins seem to show approximately the same degree of heat-resistance in both cases. Even in combination with activators the hemolytic property of heloderma venom is comparatively weak as compared to the cobra venom. Moreover, it differs from cobra venom in not being activated by that constituent of fresh serum which is apparently identical with complement. Heloderma venom can be activated by certain blood sera, but the latter then retain their activating power after having been exposed to a temperature that destroys or somehow invalidates the complement. Our knowledge concerning the finer mechanism through which the complement participates in various reactions, or is prevented from participating, is at present too indefinite to make advisable a discussion of the cause of the difference in the behavior of the two venoms; but we may mention that it may possibly depend merely on a quantitative difference in the hemolytic strength of both venoms.

Blood-sera not having an activating influence upon hemolysis inhibited it similarly as they inhibit hemolysis by soap or saponin. A certain specific relationship seems to exist between the inhibiting serum and the blood-corpuscles used; in certain cases the hemolysis of the blood-corpuscles of a certain species seems to be inhibited, especially by the serum of the same species.

A similar relationship has been apparently observed by Besredka in some other connection (Annales de l'Institut Pasteur, xv, 1901). Besredka found that sheep serum, for instance, protects only sheep corpuscles against a hemolytic rabbit serum and not the corpuscles of another species. This apparent specific relationship between serum and blood-corpuscles deserves a further study.

The discovery of Neuberg that a certain parallelism exists between the hemolytic power of certain toxic substances and their contents in lipase suggested the responsibility of the lipase for the hemolytic effect. It was therefore of interest to compare the heat-resistance of the lipolytic and hemolytic substances in heloderma venom. After heating it to 60° C. for 30 minutes, its hemolytic power is not impaired. No appreciable loss is noticeable after heating it 10 minutes to 100° C. After heating it to 100° C. for 30 minutes a great part of its hemolytic power is lost, and after exposing it to a temperature of 120° C. for 15 minutes in the autoclave it has lost all of its hemolytic power. According to Alsberg, lipase of heloderma venom is weakened after heating to 60° C. during 30 minutes; it is destroyed after an exposure to 100° C. lasting 10 minutes. The lipolytic power of venom is therefore distinctly less heat-resistant than its hemolytic power; the venom is still hemolytic after its lipase has been destroyed through heating. We may therefore conclude that lipase and hemolytic principle are not identical in the venom of *Heloderma* and probably not in snake venoms. Of course the possibility exists that after all lipase and hemolytic principle have some components in common.

We are not in a position to state definitely through what mechanism the venom of *Heloderma* causes hemolysis. Certain facts suggest, however, that lecithin and venom may increase the permeability of the erythrocytes. In the case of cobra venom von Dungern and Coca and also Manwaring believed that the venom splits the lecithin and that a splitting of lecithin is the real hemolytic agent. In the case of heloderma venom we saw that the amounts of venom and of lecithin required for hemolysis stood in inverse ratio.

It seems to me that two facts indicate that the venom-lecithin mixture increases the permeability of the erythrocytes. In the first place, I would thus interpret the observations of Bang and Overton, who found that the action *in vitro* of cobra or crotalus venom upon tadpoles is counteracted by addition of $CaCl_2$. Now, according to Jacques Loeb and W. J. V. Osterhout (Science, Dec. 15, 1911, Jan. 19, 1912), $CaCl_2$ inhibits the entrance into the cells of various salts of monovalent atoms. We may assume that it acts in a similar manner in combination with venom, but in other cases $CaCl_2$ may produce its inhibiting influence by inactivating soaps, as has been previously pointed out by Noguchi. In a similar manner I would interpret the observation of Goebel. According to him, corpuscles suspended in 0.85 per cent NaCl solution, which can not be hemolyzed by cobra venom alone, are hemolyzed if the latter are suspended in isotonic solutions of saccharose. It seems to me that this fact is similar to the results which I obtained in the course of my investigations into the influence of external conditions upon the blood-cells of *Limulus* (Folia Hæmotologica, Bd. 4, 1907; Pflüger's Archiv, 1910, Bd. 131). In this case I have shown that solutions of non-electrolytes behave somewhat similarly to H_2O and produce similar lytic changes. We should therefore expect that correspondingly they are also more favorable to hemolysis than are salt solutions.

It would be of interest to study thoroughly the various factors in their relation to hemolysis which I investigated previously in their significance for

the blood-cells of *Limulus*. I found many analogies between the action of these factors on colloids of a proteid character and on the living cells.

The venoms of snakes, as far as they have been investigated in this respect, do not only possess the power to hemolyze erythrocytes, but also other kinds of cells, especially leucocytes. In contradistinction to snake venom, venom of *Heloderma* does not possess this destructive power. No dissolving or agglutinating effect upon leucocytes is noticeable, which may even show great phagocytic power, notwithstanding the presence of venom (Tuttle). After addition of heloderma venom phagocytosis takes place *in vivo* as well as *in vitro* and apparently in undiminished strength.

After a subcutaneous injection of heloderma venom a marked increase in the number of polynuclear leucocytes takes place in the peripheral circulation. This reaction was especially marked in animals during the process of immunization, where relatively large quantities of venom could be administered. How far this reaction depends merely upon differences in distribution of leucocytes, or on an actual increase in the output of polynuclear leucocytes into the blood-vessels from the bone-marrow, has to be decided by further investigation (M. K. Meyers and Lucius Tuttle).

As previously stated, the heloderma venom has no direct hemolytic effect; it does not digest muscle, it does not destroy leucocytes, and, we may add, it has no noticeable effect upon eggs of Echinoderms.

Cobra venom and other snake venoms have a very decided lytic effect upon various kinds of cells; they even are able to destroy bacteria. In this respect heloderma and cobra venom differ very markedly, while in the manner in which both venoms affect the living animal organism there exists much similarity; both are principally neurotoxic, causing lesions in nerve-cells and exerting their lethal effect by paralysis of the respiratory center.

The similarity between certain snake venoms and the venom of *Heloderma* is also apparent in the parallelism that exists between the doses lethal for various species. Against the venom of *Heloderma* as well as of *Cobra*, *Bungarus cæruleus*, *Enhydrina valakadien* it is found that the white rat is relatively more resistant than most of the other mammals tested, but this increase in resistance does not extend to all the snake venoms. Thus, according to Fraser and Gunn (Phil. Transactions Roy. Soc., t. 202), the white rat is even more susceptible to the venom of *Echis carinatus*, which resembles in its action crotalus venom, than the rabbit or the guinea-pig. A further similarity is the following: cold-blooded vertebrates are more resistant toward cobra and certain other snake venoms, as well as against heloderma venom, than are the warm-blooded animals. Certain differences, however, exist among cold-blooded animals in regard to their susceptibility toward heloderma venom. Especially is this true of toads, which show a marked degree of resistance. During different stages of development, the susceptibility toward heloderma venom may vary. Tadpoles are undoubtedly more susceptible to heloderma venom than adult frogs. All the invertebrates which we tested were immune against the action of venom, while *Fundulus*, a salt-water fish, was susceptible.

Correspondingly, invertebrates also show no or only very slight susceptibility to cobra venom. As we mentioned above, echinoderm eggs, although not affected by heloderma venom, are affected by cobra venom. The similarity in the lethal dose of heloderma venom pro kilo animal which we found in the case of most mammalians tested is surprising indeed if we consider that a number of variable factors determining the lethal dose comes into play, and that these factors might be expected to vary in the case of different species. Furthermore, we have to consider the difference in size and number of nerve-cells in various species. It appears doubtful what we really measure in determining the lethal dose. Is it the amount of venom entering each ganglia-cell of the respiratory center? The fact that notwithstanding these complications a great similarity exists in the lethal dose pro kilo animal indicates that the amount of venom absorbed and reaching the respective ganglia-cells must be very similar in different species. In the case of the white rat, is the respiratory ganglia-cell less sensitive, or does less venom get into contact with each ganglia-cell, or are the ganglia-cells less permeable? These questions we can not answer at present. As in the case of other poisonous animals, we found *Heloderma* to be immune against very large doses of its own venom when injected subcutaneously. Whether they are susceptible to an intracerebral injection of venom, as (according to Phisalix) snakes are in the case of their own venoms, we have not had an opportunity to investigate.

In this connection an observation of A. Flühner (Archiv f. Exp. Path., 1910, 63) is of special interest. This author found that the heart of toads is not immune against the venom of *Bufo*, which is principally a heart-poison. Natural immunity of a species against its own venom here, as well as in the case of snakes, is therefore not dependent upon a lack of susceptibility to the poison on the part of those cells upon which the toxic substance principally acts. We might here also recall the fact that the erythrocytes of *Heloderma* are not immune against the hemolytic action of the venom of this animal. We may therefore conclude that the natural immunity is based on other factors. Secondary mechanisms evidently exist which protect the sensitive cells against the action of the poison.

Phisalix believed that the natural immunity of poisonous animals against their own venom depends upon the presence of antitoxin in the circulation. In the case of *Heloderma* such antitoxic substances certainly do not exist, not even in animals whose poison gland had been removed some time previously, in order to obviate a neutralization of antitoxin which might be present by the venom discharged into the blood through a process of internal secretion. There exists, however, another mechanism through which a natural immunity could be obtained. Should some provision in other organs prevent the venom from reaching the brain and acting upon the nerve-cells, the latter would be protected against the injurious effect of the venom. Such an explanation of natural immunity was suggested by Wolff-Eisner several years ago. It seemed, therefore, worth while to compare the absorptive action of the pulp of various organs for the venom of *Heloderma*, and furthermore to make a comparative test of

the behavior of the organs of different species. Other considerations made such an investigation advisable. It is necessary for a toxin to get into contact with the surface of cells before it can exert its injurious influence. One of the means through which such contact between cells and poison can be accomplished is adsorption.

Only a few of the results obtained in this study can here be referred to. We found that organs like the liver and kidney adsorb at least as much venom as the brain. In this respect heloderma venom seems to differ from certain other poisons which have been examined, as, for instance, cobra venom. On the whole, the brain closely resembles lecithin in its adsorbing power and it is very likely that the adsorptive power of the brain is due to the lipoids and not to the proteids of the brain, which latter seem, however, to adsorb some other toxic substances, as, for instance, rabies virus. Emulsions of lecithin, however, adsorb more heloderma venom than emulsions of brain-matter. In both cases, especially the small particles of the emulsified material, adsorb a great part of the venom, and it needs therefore filtration through a Berkefeld filter to determine accurately the quantity of venom adsorbed. We may conclude that conditions comparable to those found in the case of tetanus toxin, in which brain-substance has a special antitoxic power, do not exist in the case of heloderma venom. Furthermore, the combination between lecithin and venom, or brain and venom, is only a loose one, inasmuch as after an injection of the residue obtained through centrifugation the animals die, perhaps after a previous absorption and splitting of the lecithin, while after adsorption of venom by charcoal the adsorbed venom is not at all or only very slowly given off and has therefore become innocuous.

Our comparative studies of adsorption by the organs of various species point to the conclusion that the organs of *Heloderma* and of species nearly related to it adsorb venom in a larger quantity than the organs of species not as nearly related to *Heloderma*. Our experiments seem, therefore, to indicate that certain organs of *Heloderma* might be concerned in the natural immunity of this animal against its own venom. We can, however, not yet regard as conclusive this part of our investigations. Considering the large number of variable factors present in such experiments, additional investigation, especially a comparative study of the adsorption of heloderma and certain other venoms by the organs of different species, seem to be desirable in order to place this theory of natural immunity on a solid basis. On the other hand, we must fully realize that adsorption experiments with organ-pulp *in vitro* are only a very crude imitation of conditions that exist in the body after injection of the venom, and that normally functionating organs might take up and fix to themselves a very much larger quantity of venom than the organ-pulp.

Furthermore, we have to consider the fact that according to our observations blood-serum markedly inhibits the adsorption of venom even by such a strong adsorbent as charcoal. Assuming that circulating blood should act in a similar manner, adsorption of the venom in the body would be greatly interfered with.

An interesting consideration suggested by various investigators is that adsorption of the hemolyzing substance represents the first step leading to hemolysis. We furthermore know that blood-serum inhibits venom hemolysis. Does serum exert this inhibiting action by preventing adsorption of the hemolyzing agency by the corpuscles? From our results it is clear that the chemical character of the adsorbent is not the determining factor in adsorption. Carmine, kaolin, aluminium oxide, and especially charcoal are adsorbent substances. The electric charge of the adsorbing material, therefore, does, at least in this case, not seem to be the principal factor that determines the strength of adsorption.

The marked tendency of the venom of *Heloderma* to be adsorbed became apparent very early in our studies. After boiling cobra venom and thus precipitating its albuminous material, its toxic principle, which the poison of heloderma venom resembles so closely in its action, is found in the liquid part, while the venom of *Heloderma* is partly adsorbed by the coagulum produced during the process of boiling, and its tendency to be so readily adsorbed by many substances interfered markedly with its chemical analysis. Doctor Alsberg found that the majority of the methods used so successfully by Faust in his analysis of cobra and crotalus venom were not applicable in the case of heloderma venom, on account of the readiness with which it is carried down with various precipitates; but by a modification of one of the methods used by Faust for isolating the crotalus toxin, he succeeded in obtaining the heloderma venom in a state in which it no longer gave the biuret reaction, thus proving that its poisonous principle is also a substance free from proteid or only secondarily combined with it. It shows also in this respect its close relation to snake venoms.

A more extended chemical analysis of the venom of *Heloderma* was impossible on account of the great difficulty we incurred in obtaining quantities of venom sufficient for this purpose. The same difficulty prevented us from carrying out studies in immunity as far as we had planned to do. In order to produce an antivenin against heloderma venom through injection of venom into animals, relatively enormous quantities of the venom would have to be used—very much more than was at our disposal. We had therefore to content ourselves with demonstrating the possibility of active immunization of rabbits against the venom of *Heloderma*.

Precipitins are much more readily found than antitoxin in the serum of rabbits repeatedly injected with venom. If we consider the chemical character of the venom as established by Alsberg, and furthermore the fact that this precipitin produced in rabbits reacts not only with the venom but also with the blood of *Heloderma*, although less markedly, there can be little doubt that the precipitin was produced in response to the injection of certain proteid material admixed with the poison of *Heloderma* proper, and not of the venom alone. Inasmuch as this precipitin reaction is quantitatively stronger with venom than with the blood of *Heloderma*, we may conclude that the proteids of the venom gland and of the blood are not identical—a further confirmation of the chemical specificity of the different organs of the same individual.

It may also be mentioned that the formation of precipitates, which takes place when the serum of injected rabbits and venom are mixed, may possibly account for a slight decrease in the toxicity of the venom, occasionally noticed after addition of venom to the serum of rabbits immunized against venom. We may assume that this precipitate included a certain portion of the venom and thus retarded its absorption and exerted a very slight protective influence. An antivenin which, according to Phisalix, is responsible for the natural immunity of some poisonous animals against their own venom, can not be found in the blood of *Heloderma*, as we have already mentioned. Neither does the blood-serum of *Heloderma* contain any substance which fixes complement in combining with a constituent of the heloderma venom (E. P. Corson-White). Venom itself, however, is capable of binding a considerable quantity of complement in a similar manner, as has been found in the case of snake venoms. It appears doubtful whether this effect is due to the toxic substance proper or to admixed proteid material. After repeated injections of venom, symptoms of general anaphylaxis could not be observed. The sterile abscesses which appear in animals that have received frequent injections of heloderma venom may perhaps represent a local anaphylactic reaction related to the Arthus phenomenon, to which a similar reaction observed after injection of snake venom had indeed been compared by previous investigators.

In this case also the reaction is perhaps not due to the toxin proper, but to the proteid material admixed with the venom; and this reaction could therefore be in part avoided, perhaps, if the filtered fluid only were injected without the precipitate found on heating, a procedure which entails a certain loss of venom, inasmuch as the precipitate includes some toxin which has been adsorbed.

The specificity of antivenin against snake venoms has been an object of much controversy. We thought it therefore of sufficient interest to investigate the effect of Calmette's cobra antivenin upon the venom of *Heloderma*. Both the venom of *Heloderma* and of *Cobra* are essentially neurotoxic and secondarily hemolytic. Both venoms are therefore very similar in their effect upon animal tissue. On the other hand, *Heloderma* is systematically less nearly related to the *Cobra* than any of the snakes. Inasmuch as it has been maintained that even among snakes the various neurotoxins are specifically different and that cobra antivenin neutralizes mainly the venom of cobra, it was not probable that cobra antivenin would exert any influence upon heloderma venom; but our investigations leave little doubt that Calmette's cobra antivenin does exert antitoxic effect upon heloderma venom. This action is, however, very slight and naturally very much less marked than in the case of cobra venom. Our observations confirm, therefore, the belief that there exists a graded specificity, and also prove that this specificity is less limited than has been assumed by various investigators.

The difference in the heat-resistance between the neurotoxin of heloderma and of cobra venom makes it very improbable that both substances are identical, although they may be related to each other. The results of Faust, which show a difference in the constitution of ophiotoxin and of crotalotoxin, make this conclusion almost certain.

The question how far the different effects of one and the same venom depend on one toxic principle, or on the presence of multiple poisonous bodies, is a problem much more difficult to decide. There can be little doubt that not all actions of one venom are due to one single substance. We now know that some of the ferments of the venom of *Heloderma* are not at all produced in the venom-gland, but are evidently admixtures derived from elsewhere. Furthermore, there can be little doubt that the substances present in some snake venoms, causing or preventing the coagulation of the blood, are not identical with the neurotoxins or with the hemolysins of the same venom. Still more difficult to answer is the question how far other constituents of various venoms, the neurotoxin, hemolysin, and other cytolysins, and hemorrhagin are identical. The evidence we have concerning this point appears conflicting. On the one hand, Faust's fundamental investigations into the chemical constitution of venoms seemed to simplify these problems. According to Faust, ophiotoxin exerts both a neurotoxic and hemolytic action, crotalotoxin in addition a hemorrhagic action comparable to arsenic, emetin, and sepsin. The preponderance of the local effects in the case of crotalotoxin is said to be due to the greater molecular weight of this venom as compared to ophiotoxin, which increases its colloidal properties and causes it to be more slowly absorbed. On the other hand, a good deal of evidence can apparently be adduced against this view. We might especially mention the following facts:

The heat-resistance of the so-called neurotoxin, hemolysin, and hemorrhagin is not identical in the same venom. This applies to the heloderma venom, where the neurotoxin is distinctly more heat-resistant than the hemolysin. Their resistance to the effect of certain chemicals varies. The hemolytic principle can be extracted through some substances in which the neurotoxin remains undissolved. The different constituents of a venom show a different tendency to be adsorbed by certain materials. Various venoms differ in the number and quantities of the various toxic constituents which they contain. The antivenin produced through immunization with a certain venom does not neutralize all the toxic constituents of another venom. Whether the ophiotoxin and crotalotoxin of Faust are able to call forth the production of antivenin, we do not yet know.

It appears improbable that these substances could give rise to the production of antibodies, graded in accordance with the relationship of the animals from which the venoms were derived. There exists, therefore, a certain discrepancy between the results of the purely chemical analysis of the venoms and between the evidence obtained through the application of so-called biochemical methods. In weighing the evidence we have to take into consideration the fact that some of the conclusions based on the latter methods are of such a remarkable character that we may find some difficulty in accepting them. Thus studies of hemolysins and other cytolysins of snake venoms by the method of selective adsorption lead to the conclusion that a single venom contains an almost endless number of various cytolysins, and especially hemolysins. A venom would therefore constitute a mixture of poisons

of an almost incredible complexity. If, on the other hand, we should reject such a conclusion we must be aware that much of the evidence as to the difference between hemolysins and neurotoxins of a single venom is of a similar character. At present we can only point out these apparent contradictions; it remains for further studies to solve these problems.

Provisionally we may suggest that perhaps certain proteid constituents of the venom are responsible for some of these apparent discrepancies; it may be that these proteids are able to increase or diminish certain functions of the poisonous principle and that the effects of heat and certain chemicals concern rather these secondary substances than the poison proper. As far as immunization is concerned, these proteids may perhaps, to use the terminology of Ehrlich, supply a multiplicity of haptophore groups, all linked to a uniform toxophore group, which latter would represent the poisons isolated by Faust. Future investigation will also have to analyze the mechanism through which the neurotoxic effect is produced. Do we have to assume that the structure of the various body-cells is in the main similar, as far as the conditions of permeability of the outer protoplasmic layer are concerned? Are the neurotoxic and hemolytic effects produced in a similar manner? A number of observations point to the importance of a change in cell permeability as one of the factors through which the poison exerts its injurious effect, as the significant action of $CaCl_2$ and of sugar on the hemolytic action of venom already discussed indicates. Furthermore, the observations of Noguchi, that the later stages of the developing ova of *Fundulus* were more resistant to the action of venom than the earlier stages, are in agreement with my observation in regard to the action of neutral red on *Fundulus* eggs. In this case I also found the later stages more resistant than the earlier ones, and I could furthermore show that this difference in resistance depended upon a difference in the permeability of the egg-membrane for neutral red. (Elizabeth Cooke and Leo Loeb, Ueber d. Giftigkeit. einiger Farbstoffe fuer die Eier von Asterias und von Fundulus, Biochem. Zeitschrift, Bd. 20, 1909.)

That a certain venom destroys various kinds of cells through a similar mechanism is perhaps indicated in the observations of Flexner and Noguchi, who found that cobra venom which exerts a strongly neurotoxic action has also a very marked lytic effect upon other kinds of cells. This lytic action of cobra venom surpasses that of certain Viperidæ in correspondence with the stronger neurotoxic action which cobra venom exerts in the living body. On the other hand, the dissolving action of certain venoms on muscle seems to be due to a different mechanism, and accordingly we find in this case crotalus venom exerting a more powerful influence than cobra venom.

It is to be hoped that future investigations will definitely answer these questions and will thus deepen our understanding of the conditions which determine the function and death of the various cells of the animal body.

I.

MORPHOLOGY OF THE POISON GLAND OF HELODERMA.

A. Anatomy of the Poison Gland of Heloderma. By Henry Fox.

B. Structural Changes produced in the Poison Gland by Injection of Pilocarpine. By Henry Fox (with the collaboration of Leo Loeb).

C. Transplantation of the Venom Gland. By Henry Fox and Leo Loeb.

D. Solubility of the Venom Granules. By Henry Fox and Leo Loeb.

A. ANATOMY OF THE POISON GLAND OF HELODERMA.[1]

By Henry Fox.

GROSS ANATOMY OF THE POISON-GLAND.

The gross anatomy of the poison gland was studied in both the common species (*Heloderma suspectum*) and the less familiar Mexican form (*Heloderma horridum*); a considerable number of the former and one of the latter species were available. So far as this material was concerned, it showed that the general structure and relations of the gland were similar in both species—a result which is at variance with the statement of Professor Stewart (Proc. Zool. Soc. London, 1891), who asserts that in *Heloderma horridum* there is only a single opening to each gland, while in *Heloderma suspectum* there are four or five. In the single *horridum* examined I found several openings exactly as in *Heloderma suspectum*.

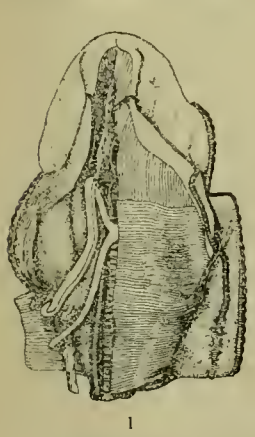

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Fig. 1.—Ventral view of a dissection of head of *Heloderma suspectum*, showing poison glands *in situ*. Dissection on right is deeper than on left. Natural size.

Fig. 2.—One-half of a dissection of the head seen from ventral side. The poison gland has been pulled outwards in order to show nerves entering its mesial surface. Natural size.

Each poison gland is of large size and is situated on the outer side of the anterior half of the lower jaw, immediately under the skin, from which it is separated by thin sheets of connective tissue. On the surface its position is indicated by a prominent swelling underlying the lower jaw.

The gland is closely invested by a capsule of fibrous tissue. From the inner surface of this capsule septa extend into the body of the gland; the larger of these septa divide the gland into three or four primary subdivisions or lobes, while the smaller, which arise in part from the investing capsule and in part from the primary septa, penetrate the lobes and there form a network dividing them into numerous lobules (see figs. 7 and 8).

[1]All the illustrations accompanying this and the following chapters were made by Dr. Fox.

The lobes are the primary subdivisions of the gland. They vary from three to four in number and are arranged in a longitudinal series. They increase in size from front to back. Each lobe is in reality a structurally independent organ, the different lobes being separated by complete fibrous partitions and opening by separate apertures to the exterior; they are bound into a compact whole by the investing fibrous capsule (*cf.* figs. 1, 3, and 4). In form they are roughly club-shaped, their upper excretory portions are narrowed, while their lower, glandular portions are rounded and swollen (figs. 3 and 4). Each lobe is a sac, containing a relatively large central excretory cavity, which narrows at its upper end to form a short excretory canal. This canal opens on the outer side of the jaw close to the base of the tooth. The wall of the sac is of considerable thickness and consists of lobules of glandular tissue. Numerous fine tubules, representing the intralobular ducts, open from the lobules into the central cavity.

The general position and structure of the gland is shown in figs. 1 and 3. It consists of four lobes, directed from above downwards and backwards.

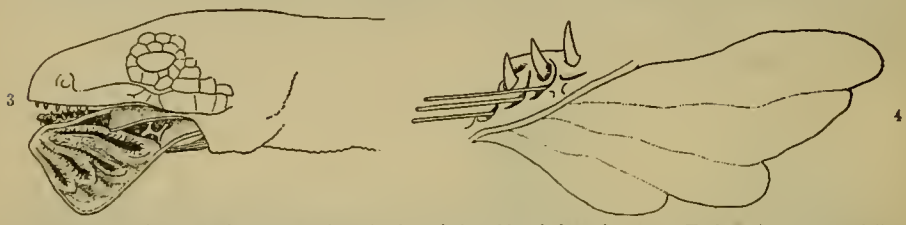

Fig. 3.—Lateral view of head, showing poison gland consisting of four independent sacs. Each sac is cut tangentially, showing its central duct or cavity. Natural size.

Fig. 4.—Enlarged view of same poison gland shown in fig. 3. Slightly diagrammatic. Designed to give a clearer view of external openings of poison ducts and their relation to the teeth. Portions of three bristles are shown inserted in the last three openings.

Their upper ends converge close to the anterior border of the lower jaw. This convergence would seem to indicate the formation of a common excretory duct, but, as already mentioned, such a common duct is not formed, each duct opening quite independently of the others, as was determined by the use of bristles and the ejection of the secretion under water. In the latter case a thin stream of fluid escaped from each opening.

As regards the course of the ducts, my observations are in accord with those of Stewart (1891) and opposed to those of Fischer (1882) and Shufeldt (1890). The last two authors described the ducts as originating from the mesial surface of the gland, passing upward through the lower jaw and opening within the mouth at the base of the teeth which they supplied. Stewart maintained that "the ducts pass directly from the gland to their openings, which are situated to the outer side of a fold of mucous membrane intervening between the lip and the jaw." Stewart's contention is undoubtedly correct. The so-called ducts of Fischer and Shufeldt are, as Stewart asserts, the branches of the inferior dental nerve which pass out through foramina in the lower jaw and enter the mesial surface of the gland (see fig. 2).

The statement of Shufeldt that the ducts open at the base of the teeth is not strictly correct. They lie, as Stewart observes, to the outer side of the

teeth, from the base of which, I may add, they are separated by the outer wall of the dental sacs. These are cup-shaped folds of mucous membrane completely surrounding the basal portions of the teeth. Immediately external to them is a shallow groove which is limited at the outer side by the prominent fold of mucous membrane mentioned by Stewart as intervening between the lip and the lower jaw. This fold is connected to the jaw by a series of vertical, obliquely transverse folds which lie in the regions between the teeth. They interrupt the continuity of the groove already mentioned and subdivide it into a series of shallow depressions, into the more anterior of which the ducts of the poison gland open. It would seem probable that these depresssions serve as temporary reservoirs for the secretion. The tips of the upper teeth project into these depressions when the mouth is closed (see fig. 4).

The teeth of *Heloderma* have been fully described by Cope (1900). Both upper and lower teeth are grooved, although no evidence of a poison gland has been found in connection with the upper teeth, and even in the lower teeth the poison ducts are associated only with the anterior three or four pairs. The latter show no features distinctly differentiating them from the remaining teeth. The grooves are formed by projecting folds of enamel which extend along both the anterior and posterior edges of the tooth. The anterior fold is the stronger and its groove correspondingly deeper. The secretion is doubtless carried along the groove by capillarity. The teeth are hollow within when dry, but there is no sign of a perforation at the apex. In life the internal cavity is filled by a fleshy papilla arising from the jaw margin. Most of the teeth are firmly united to the bone, but the younger teeth are quite free and easily detachable.

Shufeldt has described a tendinous expansion arising from the outer surface of the superficial muscles close to the hinder margin of the mandible and spreading out over the gland. Posteriorly it is rather narrow and strong, but anteriorly, as its fibers diverge, it expands to form a thin sheet closely adherent to the gland. Shufeldt is inclined to believe that, although it contains scarcely any muscle fibers, by its contraction the venom of the gland can be ejected through the ducts. As to the nature of this contraction he says nothing, but I think it may be considered as purely passive, due to the tensions produced in it by the movements of the jaw. Such movements produced artificially in the dead animal do, as a matter of fact, cause ejection of the venom. I may add to Shufeldt's account by stating that the superficial muscles from which the tendinous expanse arises are on the outer side of the posterior half of the mandible. From this point the fibers pass forward and downward and then bend inward under the lower jaw in precisely the position for the movements of the jaw to produce the tensions mentioned. In fig. 1, on the right side of the figure, this part of the tendinous expansion can be seen arising from the muscles immediately behind the angle of the lower jaw. Anteriorly it has been cut away along with the skin in order to show the poison glands.

Concerning the homology of the poison gland with the mouth glands of other vertebrates, it is obvious from its relative position that it is not com-

parable with the mammalian submaxillary, as is so frequently asserted in text-books. The latter gland opens by its duct on the floor of the oral cavity mesial to the lower jaw, whereas the ducts of the venom gland of *Heloderma* open to the external side of the jaw and in close association with the lips. From the relative position and composite structure of the poison gland, I agree with Professor Stewart in considering it as the hypertrophied representative of the sublabial glands of other reptiles and mammals.

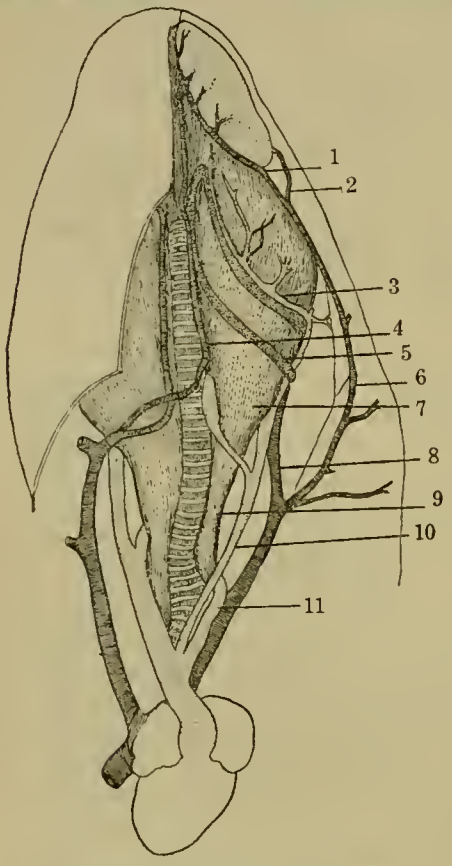

FIG. 5.—Ventral view of dissected head and neck, showing the more superficial blood-vessels. Drawing slightly diagrammatic, approximately natural size.

1. Submental vein.
2. Inferior labial vein.
3. Lingual artery.
4. Anterior jugular vein.
5. Hyoid cartilage.
6. Facial vein.
7. Pharynx.
8. Internal jugular vein.
9. Œsophagus.
10. Common carotid artery.
11. Left aortic arch.

The nerve-supply of the gland is furnished by branches from the inferior dental nerve. Four or five of these branches leave the inferior dental canal through a series of minute foramina perforating the outer side of the dentary bone; one branch, however, passes through a slightly larger opening in the articulare. The nerves enter the gland by its mesial surface, *i. e.*, the surface applied to the bone (fig. 2).

The vascular supply of the gland is derived from the inferior dental artery, branches of which pass outward through the foramina, already mentioned as transmitting the nerves, and with the latter enter the gland on its mesial side (fig. 6). Here they pass in along the interlobular septa, the ramifications of

which they follow to all parts of the organ. Most of the blood leaves the gland by a vessel which extends along its entire ventral edge. The relations of this vessel are essentially those of the submental vein of mammals. At its hinder end it is joined by the inferior labial vein. The common trunk thus formed is the facial vein. This is continued backward along the sides of the face, finally joining the internal jugular. Near its anterior end the submental vein gives off a smaller vein which curves mesially under the lower jaw and joins a vein, evidently the anterior jugular, which lies on either side of the trachea (fig. 5). Some of the veinlets in the upper portion of the gland appear to communicate with the inferior labial vein.

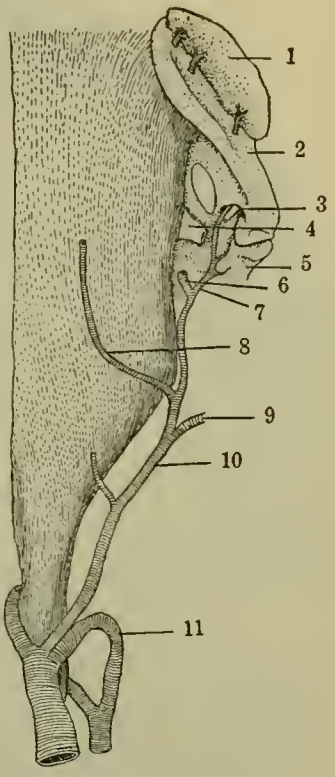

FIG. 6.—Ventral view of approximately half of head, showing arterial supply of poison gland. Drawing partly diagrammatic; approximately natural size.

1. Poison gland, turned outwards.
2. Mandible
3. Inferior dental foramen with inferior dental artery and nerve.
4. Pterygoid bone, outer half removed.
5. Quadrate.
6. Internal carotid artery.
7. External carotid artery.
8. Lingual artery.
9. Posterior auricular (?) artery.
10. Common carotid artery.
11. Left aortic arch.

HISTOLOGIC STRUCTURE.

For the minute study of the gland, pieces were fixed in various fluids, but the most satisfactory fixation was obtained by the use of Bensley's modification of Kopsch fluid. (See "The finer structure of the glandula submaxillaris" by B. A. Cohoe, in Amer. Journ. of Anatomy, vol. VI, No. 2, 1907, p. 171.) This fluid was especially favorable for the preservation of the granules in the secreting cells, the other fluids used being rather defective in this respect. The sections were stained mostly in Benda's iron hematoxylin, followed by eosin, erythrosin, or Bordeaux red. Some sections were doubly stained in safranin and gentian violet.

As already mentioned in the section on the gross anatomy, the entire poison gland is invested by a continuous capsule of fibrous tissue. From this several relatively thick septa extend inward, completely dividing the gland into three or four entirely independent parts or lobes. Each lobe is a pear-shaped sac. The center of the lobe is occupied by a relatively capacious lumen, which I shall term the central collecting duct. At its upper end this narrows gradually to form the excretory duct, which opens at the apex of the lobe. The walls of the sac are thick and formed chiefly of glandular tissue. From these walls minute intralobular tubules open into the collecting duct.

FIG. 7.—Part of section of normal poison gland, showing typical histological structure (Zeiss oc. 4, obj. 8 mm.).

Each lobe is subdivided into a large number of lobules by septa which are given off from the investing capsule and the fibrous partitions separating the lobes. These septa extend through the gland tissue as far as the central collecting duct (figs. 7 and 8). Each lobule is further cut into several smaller lobules by secondary septa which interpenetrate it in all directions, and this subdivision continues until the terminal acini are reached, each of which is separated from its neighbors by a delicate septum (figs. 7, 8, and 9).

In its general structure each lobe may be described as a compound tubular gland. The axis of the gland is formed by the common lumen of the collecting and excretory ducts. From this lumen at all levels are given off numerous smaller tubules, the intralobular tubules, which, branching repeatedly, radiate toward the periphery of the containing lobule to form the terminal acini. Topographically there is no sharp distinction between the terminal acini and the intralobular tubules; both are perfectly continuous, show approximately the same diameter throughout, and contain a wide and clear lumen. This lumen is always open; it is never obscured by the surrounding cells, even when the latter are swollen with the secretions (figs. 7, 9, and 12).

FIG. 8.—Outline drawing of a section of poison gland, showing three lobules. Drawing was made to show arrangement of inter- and intralobular septa and distribution of granule-secreting cells; the latter indicated by dotted portions of the epithelium of the tubules. The epithelium is merely outlined, none of the constituents, excepting the granules, being shown (Zeiss oc. 4, obj. 8 mm.).

In any lobule of considerable size there may be distinguished two regions: (*a*) a peripheral zone formed of more or less clearly defined clusters of terminal acini and (*b*) a central area composed of intralobular tubules. The latter converge from the periphery toward the center of the lobule and there unite to form a few central tubules which open into the large collecting duct (figs. 7 and 8).

We shall now consider in order the structure of the cells in (1) the central collecting duct, (2) the intralobular tubules, and (3) the terminal acini. In each of these parts, as previously noted by Holm, the cells are characteristic.

In the central duct the epithelium is one cell-layer thick and is thrown into a series of low undulations. In form the cells are columnar and, as Holm notices, are of slightly smaller dimensions than the intralobular tubule cells. According to Holm, there is no granulation in their protoplasm, but this is not strictly true, judging, at least, from some of my preparations—preparations which in other respects are perfectly satisfactory, and therefore probably reliable in regard to this point also. These preparations show that in nearly all of the collecting-duct cells the peripheral portion of the cytoplasm is crowded with exceedingly minute granules, the crowding being so dense that under ordinary magnification (*i. e.*, ⅛ inch obj.) the whole appears as a hyaline mass. These granules are totally unlike the large, clearly defined granules of the intralobular tubules; they are much smaller than the latter, do not stain at all with

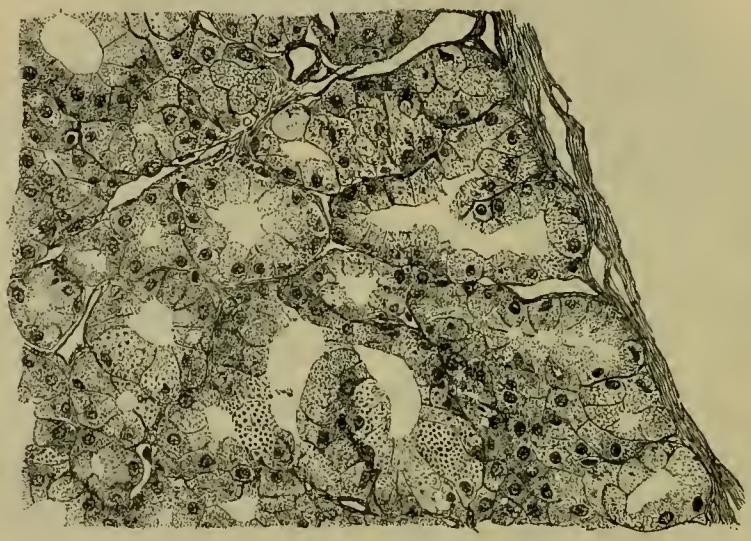

FIG. 9.—Section of portion of normal lobule, showing terminal acini, and several intralobular ducts. Note clear alveolar character of protoplasm in cells of peripheral acini and presence of granules in many cells of intralobular ducts (Zeiss oc. 2, obj. DD.).

iron hematoxylin, and absorb cytoplasmic stains only slightly. The nuclei of these cells are confined to their basal portion, where they are embedded in a rather compact mass of ordinary cytoplasm (fig. 11).

According to Holm, the central collecting-duct cells have no secretory functions. I am inclined to concur in this view, although I have observed cells which show faint traces of secreting. I have, for instance, frequently seen small granular threads extending out from the cells into the lumen of the duct, but it is possible that these may have been forced out of the cells by the pressure incident to the excision of the gland.

The cells of the intralobular tubules are columnar, but are more capacious than those of the central collecting duct (fig. 10). They are the typical granule-secreting cells. The entire inner portion of the cells bordering the lumen, including in average cases from one-half to four-fifths of their extent,

shows what I interpret as an alveolar or foam structure, the clear spaces or vacuoles being quite large and conspicuous, with only very thin strands of cytoplasm separating them. In these vacuoles lie the fully formed granules, typically a single granule to each vacuole. The granules, like those of serous glands generally, are characterized by their large size, definite form, and the avidity with which they absorb such stains as iron hematoxylin and gentian violet. In some cases the cells are so numerous and so densely crowded within the cell that they quite obscure the alveolar structure of its protoplasm. In other cases they are much less numerous and may be entirely absent. A feat-

FIG. 10.—Section passing through point of union of two intralobular ducts, showing granule-secreting epithelium. Normal gland (Zeiss oc. 2, obj. $\frac{1}{12}$).

ure which has also been noted in other glands is that while all or nearly all of the intralobular cells of a single lobule contain numerous granules, those of neighboring lobules may show few or no granules.

The mode of formation of the secretory granules appears to me to conform to the mode observed by E. Müller (Archiv f. Anat. u. Physiol., 1896, S. 317 *ff*, cited from Metzner in Nagel's Lehrbuch der Physiologie, 1907). Minute, dark-staining particles appear in the nodes of the cytoplasmic (=spongioplasmic) reticulum. These apparently increase in size and definiteness and

finally become the typical secretion granules. The latter, when fully formed, become detached from the reticulum and come to lie in the vacuoles. Some of the cells in fig. 10 show the early phases in the formation of the granules. In no case have I seen any distinct trace of filar structures within the cells, such as have been described by a number of authors in the gland-cells of the parotid, pancreas, etc.

In the process of secretion the inner membrane, facing the tubule lumen, bursts, and the secretion is extruded. Whether the granules are extruded bodily or whether they first undergo liquefaction previous to extrusion are questions which my material does not enable me to answer. I have frequently observed large, swollen, vesicle-like bodies and irregular, solid particles in the

FIG. 11.—Portion of epithelium of central duct at a point where an intralobular duct opens into it. The sections show sudden change in histological character in passing from one structure to the other. Note minutely granulose character of central duct cells and clear alveolar character of intralobular duct cells. Compare granules of central duct cells with typical secretion granules of intralobular ducts by comparing this figure with fig. 10.

gland lumen close to the ruptured inner wall of a cell, but whether these were normal appearances or artifacts due to manipulation of the excised gland previous to fixation I am unable to say. By whatever means the granules are liquefied or dissolved, they doubtless disappear as distinct structures at the time that the secretion is extruded.

A number of authors, who hold to the view that the granules are dissolved within the cell, believe that the vacuoles represent the dissolved granules. These authors maintain the reticular theory of cytoplasmic structures, according to which the spongioplasmic reticulum is composed of anastomosing threads, instead of the more viscid covering of the vacuoles postulated by the

alveolar theory. The poison gland of *Heloderma* is not a suitable object for testing the validity of the various protoplasmic structure theories, but I have chosen to interpret the facts in conformity with the alveolar theory, since on general grounds that appears to me to have most in its favor. The vacuoles appear to me to be always present, although in the empty gland-cells they are frequently quite minute, owing apparently to the shrinkage of the cells and the consequent condensation of its cytoplasm. They are present and of full size in many cells in which the early stages of granule formation, as evidenced by the presence of the minute particles in the nodes of the reticulum, were alone visible. I am inclined, therefore, to view the vacuoles as relatively constant

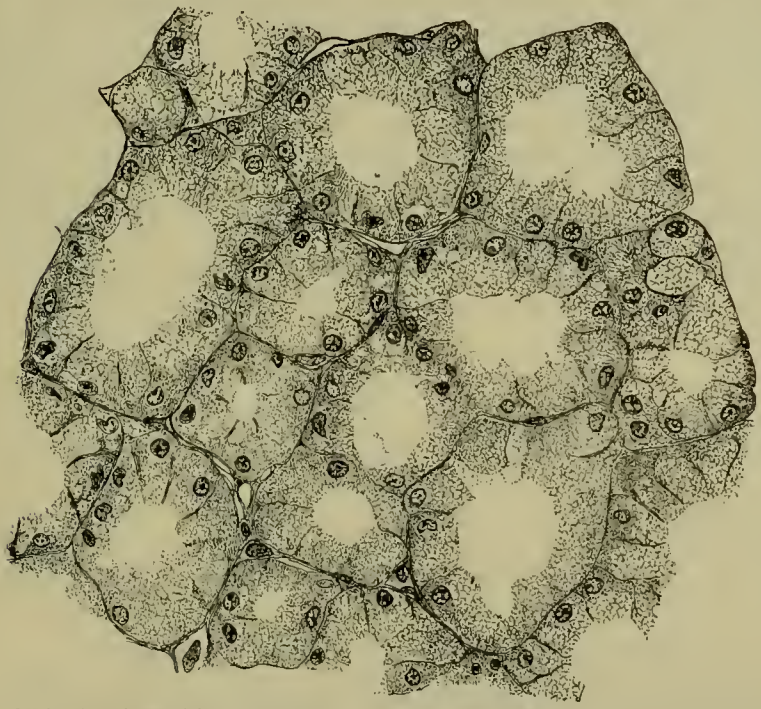

FIG. 12.—Section through part of single lobule of poison gland, showing terminal acini (Zeiss oc. 4, obj. 3 mm, ap. 0.95).

constituents of the cell protoplasm. Whether at the moment of secretion the granules dissolve in the liquid of the vacuoles, or are instead extruded into the gland lumen, where they quickly dissolve, can not be settled by the material examined.

The outer or basal portions of the granule-forming cells are filled by a relatively dense, homogeneous cytoplasm, similar to that which nearly all authors have described for that portion of the gland-cell. The protoplasm appears to be alveolar, but the vacules are extremely minute and closely crowded together. The nucleus is embedded in this cytoplasm.

The nucleus of the gland-cells is typically quite large and conspicuous, and, as already mentioned, is embedded in and surrounded by a relatively dense

perinuclear cytoplasm occupying the basal portion of the cells. In form it is spherical or elliptical, but I have observed no constant relation between the form of the nucleus and the secretory phase of the cell. Shrunken nuclei were observed in cells entirely filled with granules, but in all other cases the nuclei were of approximately the same average size. I have not observed in *Heloderma* the changes in the nucleus described by Matthews in the pancreas and by Launay in the poison gland of the viper, as associated with the changes in activity of the cell. The nuclei of the active cells, as far as I have observed, show no constant differences from those of the inactive cells.

The cells of the terminal acini (fig. 12) are slightly smaller than the intralobular duct cells. In form they approximate to the cubical type. Their cytoplasm is distinctly alveolar, this structure being clearest on the side facing the lumen. At the base it is much denser, resembling that in the corresponding part of an intralobular tubule cell. The large secretory granules, so evident in the latter, are entirely lacking in these cells, though minute granular particles are rather thickly scattered through the cytoplasm. The interspaces of the reticulum are occupied by a clear ground-substance, which failed to stain with the usual mucus stains. The nuclei showed no constant differences from those of the intralobular duct cells.

B. STRUCTURAL CHANGES PRODUCED IN THE POISON GLAND BY INJECTION OF PILOCARPINE.

By Henry Fox.

(*With the collaboration of Leo Loeb.*)

In order to study the different stages of activity of the poison gland of *Heloderma* under various conditions, the animals were injected subcutaneously with pilocarpine. The general effects of the pilocarpine were strikingly similar to those described in the case of salivary glands. (See Cohoe, Jour. of Anat., vol. VI.)

Experiment I.

An animal (No. 1) was for the first time injected with 0.1 grain of pilocarpine. Immediately before the injection a portion of the poison gland was removed and fixed in Kopsch fluid to serve as a control. The animal was then injected and three pieces of the gland were removed at intervals of 15, 35, and 50 minutes, respectively.

(1a) Gland Removed Previous to the Injection.

This was found upon microscopic examination to be entirely normal. Numerous granules were observed crowding the cells of the intralobular tubule, the undifferentiated cytoplasm being limited to an extremely thin layer at the base of the cells. A copious granular secretion was found in the lumina of the tubules, but it is doubtful if this condition is normal—it may have been forced out of the cells into the tubules as a result of the handling consequent upon the removal of the gland.

(1b) Gland Removed 15 Minutes after Injection of Pilocarpine.

Sections showed most of the intralobular duct cells devoid of granules, though a moderate number retained a few. As a rule the cells presented the usual character of stimulated gland-cells, being quite or almost free of granules and with large, clear vacuoles inclosed in the meshes of the cytoplasmic reticulum. Within the tubules the amount of secretion visible was extremely small, in marked contrast to the quantity observed in the normal gland. If the latter condition was normal, one evident effect of the stimulation with pilocarpine was a rapid solution of the extruded granules or an expulsion of the secreted material from the gland proper. One remarkable feature of pilocarpine stimulation shown in this case is the extreme rapidity of its action as compared with its action on salivary glands. According to Cohoe, the submaxillary gland of a rabbit showed all the granule-cells loaded with granules after 3 hours' stimulation with a dosage of 0.014 gm. per kilo of body-weight of the animal.

(1c) Gland Removed 35 Minutes after Injection of Pilocarpine.

The appearance of this gland was very similar to the preceding, the chief difference being that the number of intralobular duct cells containing granules was considerably less and that the granules were fewer in each cell; but in one place near the cut surface of the gland granules were observed in abundance. In the lumina of the tubules a moderate quantity of the granular secretion was present, the amount being slightly more abundant than in 1B.

(1d) Gland Removed 50 Minutes after Injection of Pilocarpine.

Granules appeared to be almost entirely absent in the intralobular duct cells, only a few cells in the more distal portions of the tubules showing any clear trace of them. The greater number of these showed the granules in what appears to be an incipient stage of formation, namely, as dark-staining nodular thickenings of the cytoplasmic reticulum. The majority of the granules were minute, but some were larger and closely resembled the typical granules. Frequently the granule-containing cells were observed to be much swollen, the adjoining cells, which had not yet begun to reform the granules, being compressed into a narrow space.

A few cells were observed each containing two nuclei, usually in process of disintegration; but such cells were excessively rare, and, owing to the difficulty of finding and of clearly identifying them, it is doubtful if they are normal constituents of the gland. They are probably not a result of the pilocarpine stimulation.

In the lumen of the tubules a granular secretion was present, but less in amount than in the unstimulated gland. At certain places in this secretion were observed many apparently pycnotic cells and nuclei. Whether these were desquamated into the lumen in the normal process of secretion or were merely detached as a result of the pressure to which the gland was subjected in the process of removal from the animal we are unable to solve through a study of the sections.

Experiment II.

An animal (C) was injected with 0.1 grain of pilocarpine, a portion of the unstimulated gland having been previously removed and fixed in Kopsch fluid as a control. The animal was then injected and 10 minutes after the injection another portion was removed and fixed.

(C1) Normal, Unstimulated Gland.

This gland appeared to be normal, though in many of the tubules the cells, or a considerable number of them, were devoid of granules; but such a condition is not unusual for normal glands, as shown, for example, by the series of sections on which the description of the normal gland was based and in which many of the lobules were mentioned as showing only a few granule-containing cells, while in others the cells were crowded with granules. A similar condition is shown by this gland. In many lobules numerous cells containing the granules were observed, while in others the cells were empty.

(C²) Gland 10 Minutes after Injection of Pilocarpine.

Sections showed the granules still present in a considerable number of cells, but the latter were less numerous than in the unstimulated gland. The number of granules in each cell was also less. The lumen of the tubules contained an abundant granular mass.

Experiment III.

An animal (D) was injected with 0.1 grain of pilocarpine and, after an interval of 24 hours, pieces of the gland were removed and fixed in Kopsch fluid. Examination of the sections indicated that the gland had almost regained its normal activity. Some of the lobules were apparently perfectly normal, the

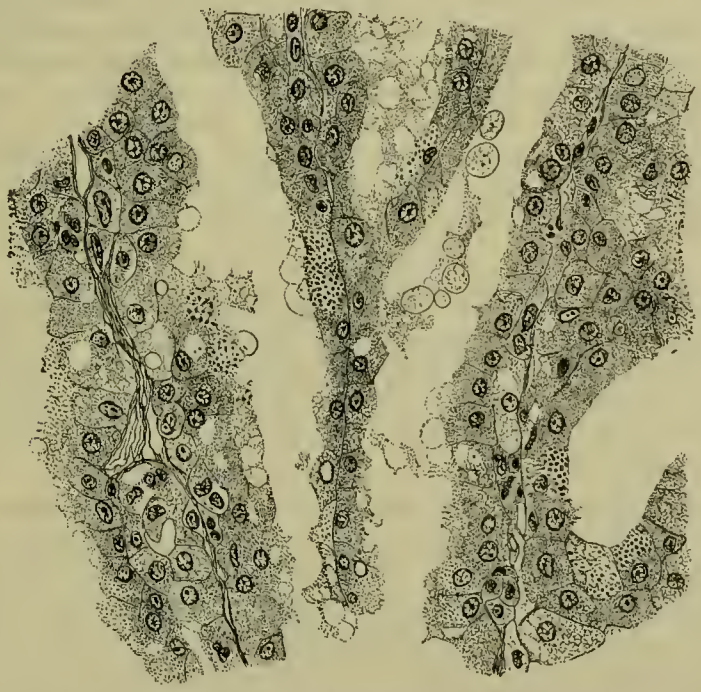

Fig. 13.—Two thin sections of intralobular tubules of a gland 24 hours after injection of pilocarpine. Some cells show initial steps in granule formation (Zeiss oc. 4, obj. 3 mm., ap. 0.95).

number of granule-containing cells being nearly if not quite as numerous as in similar lobules of the normal gland. In other lobules the number of such cells was much less and in many instances they contained only a few granules. In many cells the granules were apparently in the process of formation, appearing as minute particles at the intersections of the cytoplasmic network.

Fig. 13 represents a section passing through two intralobular tubules. The greater number of cells, it will be observed, are filled with a relatively dense cytoplasm similar to that which in normal granule-containing cells forms a basal layer (*cf.* fig. 10). In other cases the cytoplasm is more clearly alveolar. In these cells granules are usually present, in some cases in early stages of formation, in others almost fully formed.

Compared with the cells of the normal tubules, the cells of the gland, after injection of pilocarpine, appear much shrunken. The free surface of the cells also appears to be less regular, though in neither case have we been able to observe a limiting membrane, the naked protoplasm appearing to be in direct contact with the lumen (*cf.* fig. 13).

A small amount of secretion was present in the tubules. This was similar in character to that observed in other glands, both normal and stimulated. As shown in fig. 13, it consisted of a finely granular matrix, in which were embedded a number of relatively large vesicular masses. These bodies are cells apparently detached from the epithelial lining. They contain a finely granular cytoplasm which shows in some cases a clear alveolar and in others an irregular structure. Occasionally, as shown in fig. 13, a few small secretion granules may be found in the vesicles. In the figure nuclei are not shown in these bodies, but in other sections the nuclei, usually much shrunken, have been seen. Sometimes free nuclei occur in the secretion.

In the same figure some of the cells are seen giving off a clear secretion which, as it leaves the cell, assumes the form of a vesicle. In the lumen, however, as indicated in one part of the figure, these vesicles soon lose their regular outline and disintegrate.

Experiment IV.

An animal (B) was injected with the usual dosage of pilocarpine and on the following day the injection was repeated. Two days after the first injection the animal was again injected, making altogether three injections at intervals of 24 hours. Immediately before the last injection a portion of the gland was removed and fixed in Kopsch fluid. A second portion was removed and fixed 15 minutes after the injection.

(B^1) A Gland Stimulated on Each of the Two Preceding Days.

This gland was removed immediately before the third injection. After having been twice stimulated at intervals of 24 hours, it had been given for 24 hours opportunity to recover from the effects of the stimulation. Examination of the sections showed that it had done this very imperfectly. The granules were found to be rather numerous in the cells of a few lobules, but they were either entirely lacking or exceedingly scarce in most of them. The lumen of the tubules was found to contain a considerable amount of secretion.

(B^2) Gland 15 Minutes Following Third Injection of Pilocarpine.

Careful examination of the sections failed to reveal granules in any part of the gland. The ducts contained considerable granular material in which disintegrating cells and vacuolar vesicles were scattered.

Experiment V.

Simultaneously with the preceding animal, another individual (C) was injected on three successive days. The third injection was made with 0.125 grain of pilocarpine. One part of the gland was removed previous to this

injection and 17 minutes after the last injection of pilocarpine another part was removed. This experiment gave results similar to Experiment IV, the chief difference being the amount of secretion present in the lumen of the tubules. In the present instance this secretion was scanty, whereas in B it was quite abundant. In that part of the gland removed previous to the third injection, the cells containing granules were very few and were found in widely separated parts. In the part removed 17 minutes after the third injection, no granules were seen in any part.

CONCLUSIONS.

The experiments with pilocarpine warrant us in drawing the following conclusions:

(1) Injection of pilocarpine in doses of 0.1 grain begin to act almost immediately upon the poison gland, causing within a period of 15 minutes an almost complete disappearance of the secretion granules from the intralobular duct cells.

(2) Recovery of the gland from the effects of a single injection of pilocarpine is noticeable as early as one hour after the injection, and is indicated by a swelling of the intralobular duct cells and a formation within them of minute thickenings of the cytoplasmic reticulum.

(3) Full recovery from the effects of a single injection would appear to require at least an interval of 24 hours.

(4) Two injections of the usual dosage of pilocarpine, the second injection following the first after an interval of 24 hours, appear to exert a stronger action upon the gland, as after 24 hours' rest a gland so stimulated shows only a partial recovery from the effects of the injection.

(5) Two injections of pilocarpine, carried out as mentioned in the preceding paragraph, appear to have no effect upon the ability of the gland to respond to the action of pilocarpine. A gland injected twice with the usual dose of pilocarpine responded just as readily as a normal gland to the action of the drug after 24 hours' rest, although the gland had only imperfectly recovered from the first two injections of pilocarpine.

C. TRANSPLANTATION OF THE VENOM GLAND.

By Henry Fox and Leo Loeb.

With one exception all the experiments mentioned under this heading were carried on in the early summer of 1909. Two series of transplantations were planned. In the first series a portion of the gland was cut out and transplanted to the right side of the thorax of the same animal, where it was inserted within the muscles just underlying the skin. In the second series another portion of the same gland was transplanted to a similar place on the left side of a different individual. The transplanted glands were then removed after intervals of one, two, three, and four weeks, respectively, fixed, and sectioned. In every case where a transplanted gland was removed, a piece of the normally situated injured gland and one of the uninjured gland were removed and studied as controls.

Series I.

Experiment A.—Gland Examined One Week after Transplantation.

The animal H was operated upon on June 4. A portion of the right gland was cut out and divided into two pieces. One piece, designated H^1, was transplanted to the right thorax of the same individual; the other, designated K^2, was transplanted to the left thorax of another animal, K.

One week later (June 11) the piece H^1 was removed and fixed in Kopsch fluid. Previous to fixation a small section was cut off and transplanted into an albino mouse, which died a day or two later. Examination of the gland showed the central area completely necrotic, the entire mass being composed of an irregular aggregation of opaque, more or less hyaline particles, among which were scattered numerous, small, densely-staining granules representing necrotic nuclei. Surrounding the necrotic area more or less completely was a middle zone of pycnotic elements. In this zone the individual cells were recognizable, but much shrunken; their nuclei were shriveled and closely pressed against the basement-membrane.

The peripheral portion consisted in part of an extensive zone of nearly normal gland-tissue. In this zone the terminal acini were recognizable, but their typical structure was obscured by a process of desquamation whereby the central lumen had become largely filled with necrotic cells. The living cells of the acini were quite large and apparently normal, except that the cytoplasm was less clearly alveolar in structure and tended to collect in minute droplets, thereby giving the cells a decidedly granular appearance. As a rule, the nuclei were also more shrunken than those of the normal gland-cells. In many cases the chromatin was densely aggregated throughout the entire nucleus; in

other cases it formed discrete particles or knots, the remainder of the nucleus being nearly clear. None of the cells on this gland were observed to contain typical secretion granules.

Experiment B.—Gland Examined Two Weeks after Transplantation.

On the same day that animal H was operated upon, another individual, K, was also operated upon. Its right gland was exposed and a portion removed. This was then divided into two pieces, one piece, K^1, being transplanted to the right thorax, while the other, H^2, was transplanted to the left thorax of H.

Two weeks later, June 18, the former piece, K^1, was removed and cut into two pieces. The larger piece was fixed in Kopsch fluid, the smaller transplanted to an albino mouse, which died shortly afterwards. In this gland the same zones were observed as in the preceding, *i. e.*, a superficial zone of nearly normal gland-tissue, a middle zone of pycnotic elements, and a central area of necrotic material.

The area occupied by the apparently normal gland-tissue was considerably larger in this than in the preceding, H^1, and it had a much more healthy aspect. In H^1 this area formed a very thin zone, while in K^1 it formed nearly a third of the entire transplanted piece. In some of the tubules and acini a large and distinct lumen was present, quite normal in every respect, and in some of the cells of the tubules the typical secretion granules were observed, whereas in the transplanted piece, removed one week after transplantation, no trace of these was evident. In many of the tubules and acini the lumen was more or less occluded by dead and desquamating cells in a manner similar to that found in H^1. The nuclei of these cells in the peripheral tubules were apparently quite normal, in strong contrast to those of H^1, which were nearly all shrunken. Some of the nuclei of the gland K^1 presented the same shrunken aspect, but they were the exception and not the rule.

The middle pycnotic zone was variable. Those cells lying nearest the normal layer were rather indefinitely outlined, but had nuclei of nearly normal form and size, with a clear ground-substance and somewhat scattered chromatin particles. The deeper cells were more degenerated, with small shrunken nuclei in which the chromatin was massed in lumps.

The necrotic portions had no special features to warrant description. It was similar in every respect to the corresponding portion in H^1.

Experiment C.—Gland Removed Three Weeks after Transplantation.

An animal (M) was operated upon on May 31. As in the other cases the right gland was exposed and a portion of it removed. One half, M^1, of this was then transplanted to the right thorax, while the other half, N^2, was transplanted to the left thorax of another individual, N. Three weeks later the transplanted gland M^1 was removed. Part was fixed in Kopsch fluid and the remainder transplanted to a mouse, which died within an hour after the operation.

As shown in typical sections, this gland appeared to consist of equal parts of normal and necrotic elements. Toward one side all the lobules were normal;

toward the other they were necrotic. A few lobules near the center were intermediate, being in part necrotic, in part consisting of normal gland-tissue.

In the living lobules the cells were in general large and quite irregular, differing in no visible respect from normal gland-cells. In most of the living lobules the cells appeared in good condition; in some, especially those near the center, a narrow zone of pycnotic cells intervened between the living cells and the necrotic mass. In many of the lobules the tubules and acini showed a large and distinct lumen which contained a mass of more or less translucent particles and numerous desquamating cells or cell-groups.

The cytoplasm in the living cells was moderately dense and finely reticulate with minute granules. But no typical secretion granules were found in them.

In some acini indistinct mitotic figures were observed, but were very scarce.

In the intermediate zones there were numerous dying cells. Some of the cells were apparently living, but showed no distinct outlines or regular structure. Other cells were entirely vacuolar, owing to liquefaction of their contents. Many of their nuclei were large and vesicular, with an almost colorless ground-substance and scattered chromatin knots.

In the necrotic portion of the gland were observed numerous connective-tissue cells apparently invading it from all directions. Their nuclei were rather regular in outline, sometimes rounded, sometimes elongated, and occasionally constricted on one side. Each showed a clear ground-substance through which were scattered numerous fine chromatin particles, while part of the chromatin was aggregated near the center into a conspicuous nucleolus-like mass.

Experiment D.—Gland Removed Four Weeks after Transplantation.

A fourth animal (N) was operated upon on May 31. A portion of its right gland was removed and divided into two pieces. One piece, N^1, was then transplanted to the right thorax of N; the other, M^2, was transplanted to the left thorax of M. The gland N^1 was removed June 28 and fixed in Kopsch fluid. A part of it was transplanted to a mouse, which died after a few days.

This piece of gland was more nearly like the normal gland than any of the other transplanted pieces removed at earlier periods. The peripheral lobules were all quite normal in appearance. Thus in one lobule selected for study the terminal acini were to all appearances quite normal and constituted the bulk of the lobule. Each acinus was separated from adjacent acini by clear fibrous septa, similar to those separating normal acini. The cells were typical in form and contained an alveolar cytoplasm in which coarse granulations were present at the nodes of the reticulum. The nuclei were situated close to the basement-membrane, and in many cases were rather more shrunken than normal nuclei. This shrunken condition was not, however, pronounced, while in other cases the nuclei were apparently quite normal. Each nucleus contained a clear nuclear sap in which were scattered grains of chromatin and a central chromatin knot, the entire chromatin arrangement being essentially like that

observed in normal nuclei. In this lobule, the lumina of the smaller acini were either empty or contained only an insignificant amount of a coarsely granular mass. In the larger acini or tubules this mass was much more copious. In some cases, especially in the largest tubules, it also contained free nuclei, which in one instance were observed aggregated in a dense cluster in the center of the tubule.

In the lobule just below the one described in the preceding paragraph, but in a deeper part of the gland, the conditions deviated somewhat from the normal. In this lobule the intralobular ducts were numerous and in most cases of relatively large diameter. They contained a granular mass with occasional desquamated cells or nuclei. They were lined by a single layer of epithelium which, in those tubules or those parts of a tubule nearest the surface of the gland, consisted of either columnar or cubical cells, while in the deeper tubules or deeper parts of a larger tubule the cells were much flattened. In that portion of the lobule nearest the surface of the transplanted piece, the various tubules and acini were separated by clearly defined fibrous partitions, but in the deeper portion of the lobule these were less distinct and were largely replaced by solid aggregations of rounded or spindle-shaped cells. Usually these cells were clustered about the tubules and were not always clearly separable from the epithelial lining of the ducts. A mitotic figure was observed in one of these cells. The origin of these cells we have not been able clearly to determine. They may possibly be derived from the proliferating epithelium of the tubules, their usually close association with the latter favoring this view; on the other hand, they may be derived from proliferating connective-tissue cells.

Since these cell-aggregations largely grouped themselves about the various tubules, there were left between them narrow, clear spaces with indications of a very loose connective tissue through which were scattered minute granules of apparently necrotic material.

In another lobule lying to one side of the one last described, the conditions were still less normal. The superficial part of the lobule alone showed tubules with a distinct lumen; in the remainder of the section the outlines of the original tubules were rather indistinctly indicated by clusters of rounded cells, the cells of each cluster being grouped more or less concentrically around the center, which in one case at least showed signs of a lumen. These clusters were observed to be separated by an anastomosing network of loose connective-tissue septa in which necrotic particles were abundant and in which numerous elongated or spindle-shaped cells were scattered.

In the deeper parts of this lobule most of the solid cell-clusters were very small—considerably smaller than any tubules observed in normal glands. A number of these appeared to be grouped in a radial manner about a common center and invested by an indistinct layer of necrotic connective tissue. These masses appeared to correspond to single tubules, the solid cell-mass of the latter having degenerated into a number of cell-clusters between which connective-tissue cells then penetrated.

One lobule, which was situated beneath the superficial zone, showed three well-defined areas in which the tubules were in different conditions. In the more superficial area there was a fairly broad cap of clearly defined tubules and acini, in each of which a large lumen was present. These tubules were separated by thin, fibrous septa, which, however, were much more nearly normal in the superficial part of the zone than in the deeper part, where the fibrous elements were largely necrotic and partly replaced by thin layers of elongated cells. The epithelial lining of the tubules was not entirely normal, the cells showing considerable shrinkage, with loss of clearly defined outlines, in many instances. Each tubule in this zone contained a considerable amount of granular material through which, in some cases, were scattered a few nuclei.

Below this superficial area came a middle zone of the lobule which was almost entirely occupied by four exceptionally large vesicle-like tubules. These tubules were considerably larger than any of the normal intralobular tubules. They may perhaps have been formed by the fusion of two or several adjoining tubules as the result of the degeneration of the intervening septa and of the desquamation of some of the epithelial elements. Each was lined by a single layer of greatly flattened cells, resembling very much the epithelium of the lung alveoli. These large tubules contained considerable granular matter, in some cases with, in others without, desquamated cells and nuclei. In the largest tubules these desquamated cells were collected into two compact masses, one situated in the center of the lumen, the other to one side, close to a point where the main body of the tubule opened widely into a smaller vesicle. In the case of the latter the origin of such masses by the aggregation of desquamated cells was clearly shown, one cell of the mass with its nucleus being observed detaching itself from the epithelial wall.

The deepest zone of this lobule consisted of several circular or ovoid solid masses of round cells, each mass being separated from its neighbors by a septum formed of rather loose connective tissue.

In this gland mitotic figures were observed in fair numbers, indicating a rapid proliferation of the cellular elements of the gland.

Regular, clearly defined secretion granules were not abundant in any of the lobules, even in the almost normal ones near the surface, but they occurred in one or two of the latter, and in the cells of some of the tubules were almost as abundant as in normal cases.

The lobules in the interior of the gland were largely necrotic, but frequently one or several tubules were preserved or were represented by solid cellular masses, similar to those already described. The necrotic material, however, was much less dense than in the transplanted glands already described, and interspersed amongst it were numerous apparently normal cells, some clearly rounded, others ovoid or spindle-shaped, of connective-tissue origin. We see, therefore, proliferating connective tissue substituting part of the necrotic material.

EXPERIMENT E.—GLAND EXAMINED TWO MONTHS AFTER TRANSPLANTATION.

It was considered advisable to observe the changes produced in the gland after a longer period of transplantation. Accordingly, on January 18, 1910, we removed a piece of the right poison gland of a *Heloderma* and inserted it into the same side of the thorax of the same animal. On March 18 the transplanted gland was removed and fixed in Kopsch fluid. Typical sections showed almost the entire gland to be composed of fibrous connective-tissue through which were distributed numerous connective-tissue cells, mostly spindle-shaped. In most cases the connective tissue of the different lobules had so completely fused as to form a nearly homogeneous mass, in which it was almost impossible to recognize the outlines of the original lobules, tubules, or acini. Remnants of the gland were, however, present in the form of large

FIG. 14.—Section of peripheral part of a transplanted gland removed from the thoracic region of a *Heloderma* one week after transplantation. The nearly normal living elements of the peripheral portion and the pycnotic and necrotic mass of the deeper portion are shown (Zeiss oc. 4, obj. AA).

vesicles, similar in all essential respects to the large vesicle-like tubules described under Experiment D. Some of the vesicles were close together, but others were separated by broad partitions of connective tissue. The cells of the vesicles were mostly rather flat, but some of them were cubical or columnar.

Closely associated with one group of these vesicles were several tubules of normal size and approximately normal appearance. Other tubules or follicles of small size were observed scattered through the general fibrous tissue, but were few in number. A considerable part of the transplanted gland-tissue at some distance from the vesicles just described was entirely necrotic. Along its edge next to the connective tissue the necrotic material was being replaced by ingrowing masses of connective-tissue cells.

Series II.

In this series a portion of the poison gland of one individual was excised and transplanted to the left side of the thorax of a different individual.

Experiment A.—Gland Removed One Week after Transplantation.

On June 4 a part of the right poison gland of individual K was transplanted to the left thoracic wall of H. On June 11 this was removed and fixed in Kopsch fluid. A portion of this gland is shown in fig. 14. In its general features sections of this gland resembled those of H^1, as described under Series I. The chief difference was in the size of the intermediate pycnotic zone, which was either very narrow or absent in this gland. The periphery of the gland consisted largely of a relatively broad zone of apparently normal gland-tissue, aggregated into tubules and acini, all of which were provided with clear and distinct lumina.

The inner necrotic portion formed fully three-fourths of the entire mass of the transplanted piece. Here the various lobules and their subdivisions were distinctly visible, and even the lumina of the tubules were recognizable, but the cellular elements were fully necrotic. No typical secretion granules were observed in any of the cells of this gland.

Experiment B.—Gland Removed Two Weeks after Transplantation.

This piece of gland was obtained from individual H on June 4 and was transplanted to K, from which it was removed on June 18. This piece, designated K^2, was nearly normal throughout. Only in the very center of the section was a relatively small necrotic area. The cells of the approximately normal portion had the usual size, but their cytoplasm appeared rather more granular than normal. The typical secretion granules were also present, but were confined to the intralobular ducts of a few lobules. In this specimen the nuclei, even of otherwise apparently normal cells, appeared to be much shrunken.

As mentioned above, the necrotic area was confined to the central part of the transplanted piece. In places the pycnotic and necrotic elements were inclosed by an investing sheath of fibrous tissue. Usually the tubules of these areas were distinct, but in most cases they lacked a distinct epithelial lining and their lumina were greatly occluded by large quantities of desquamated cells and necrotic matter.

In all tubules, both normal and pycnotic, there were large accumulations of desquamated cells.

Experiment C.—Gland Removed Three Weeks after Transplantation.

This piece of gland, M^2, was obtained from individual N on May 31 and transplanted to M, where it was allowed to remain until June 21, when it was removed and fixed in Kopsch fluid; part of it was transplanted to a mouse just after the second removal, the mouse dying within a few days. M^2 was rather less normal than piece K^2, removed two weeks after transplantation, and in many respects closely resembled the piece H^2, removed after one week. Most of the peripheral portion, forming about one-third of the total mass,

consisted of apparently normal tissue aggregated into acini and tubules, each with a clear and distinct lumen, the latter usually containing more or less granular and desquamated material. The cells in general were regular in size, structure, and contents. The typical secretion granules were mostly absent, but in one lobule they were observed in profusion, the cells in which they were found having the appearance of normal intralobular duct-cells. In the deeper portions of the peripheral zone the cells were not so regular and the lumina of the tubules were less distinct. The interior of the piece consisted entirely of necrotic material.

Experiment D.—Gland Removed Four Weeks after Transplantation.

The gland used in this experiment was obtained from individual M on May 31 and was transplanted to the left thorax of individual N. There it remained until June 28, when it was removed and fixed in Kopsch fluid. A small piece of it was at the same time used to inject a mouse, which died shortly afterwards. The most prominent feature shown by this gland was an extensive proliferation of small round cells, either of connective-tissue origin, or leucocytes emigrated from the blood-vessels. These cells were exceedingly abundant about the periphery of the gland, where they formed a dense layer of considerable average thickness. Beneath this layer was a zone, not very thick, of gland-tissue, which, however, was in most lobules not entirely normal. In one or two lobules some of the acini seemed to be entirely normal, each with a clear lumen, but usually the cells were more or less shrunken, while the lumina were either indistinct or filled with desquamated cells or disintegrated material. Here and there in these same lobules some of the larger tubules showed a distinct lumen, but the cells lining it were much more flattened than in normal cases.

The small round cells mentioned above extended into the lobules along the septa. The latter showed only slight traces of fibrous tissue, and were almost entirely composed of strands of round cells. In the deeper layers of the transplanted piece, where the acini were mostly pycnotic, the round cells spread out beneath the lobule, frequently forming dense masses aggregated about the dying gland-cells or congested blood-vessels. From these points the small cells were found to radiate into the subjacent necrotic areas.

The necrotic region of the transplanted piece was very extensive. Compared with the same region in the transplanted pieces removed at an earlier period, it was distinguished by a great reduction in the amount of actual necrotic material. In the earlier stages the latter was so abundant as to make the necrotic regions more or less strongly opaque. In the present gland, on the other hand, the actual quantity of purely necrotic material was small, the entire region consisting almost exclusively of a loose fibrous tissue, with large, clear interspaces and numerous widely scattered round cells.

In a few places the round cells were observed to form dense strands extending from the periphery into the necrotic portions. Usually, however, the infiltration appeared to be irregular, the cells dispersing in all directions from the aggregations at the base of the lobules.

None of the typical secretion granules were observed in this gland.

Series III.

In Series III portions of those glands from which the pieces transplanted in the preceding experiments had been cut were examined. They were used partly as control and partly to determine whether regeneration of the excised portions took place. For the latter purpose the sections were made at right angles to the superficial blood-clot which filled the cavity produced by the excision. Examination of the sections showed the glands to be normal in every respect. No sign of regeneration of the missing parts of the gland was observed. Measurements of the cells showed that no perceptible shrinkage or hypertrophy had taken place.

Series IV.

In Series IV pieces taken from the normal, uninjured gland of the animals in which the gland of the other side had been partly excised were examined. They were used as controls. These glands were perfectly normal in every respect. Typical cells were measured and were found to have the average size of cells of glands taken from animals that had never been operated upon.

Series V.

Only one experiment was made in Series V. A part of a normal gland, taken from Gila monster N, was transplanted to a turtle. The latter died 3 days later. The transplanted piece was then removed and fixed in Kopsch fluid. Microscopic examination of this piece showed the superficial portions apparently quite normal. The cells were of the average size and their contents, although somewhat granular, were not much more so than may be seen frequently in preparations of the normal gland. In the intralobular duct-cells the large, typical secretion granules were often present, sometimes quite filling up the entire space within the cell. There was very little desquamation in this region. The deeper lobules were much disorganized, although the form of the individual tubules and acini had been fairly well preserved. The elements were pycnotic and there was much desquamation, the lumina containing large quantities of both disintegrated material and necrotic cells and nuclei.

SUMMARY.

(1) Pieces of the poison gland, transplanted to the muscles and subcutaneous regions of the thorax, continued living in their peripheral portions for a period of at least a month, while in one case a few tubules were observed alive after an interval of 2 months. The central portions of the transplanted glands always undergo necrosis. In the course of from three to four weeks the necrotic material is largely absorbed and its place taken by connective tissue.

(2) There appears to be no marked difference between the pieces transplanted into the same animal from which the piece of the gland had been excised and those transplanted into other individuals of the same species.

(3) The transplanted glands show little, if any, power of regeneration. The cells of individual tubules may, however, divide by mitosis and replace the necrotic cells of the same tubules, but any further regeneration apparently does

not take place, although an extensive desquamation of cells takes place in the tubules and acini of the transplanted glands.

(4) In the living portions of the transplanted pieces, the regular secretion granules were observed in those pieces which had been removed two, three, and four weeks after transplantation, none being seen in those removed a week after the operation. The transplanted glands retain, however, their toxic properties at all times, as shown by the fact that when pieces of them are used to inoculate mice the latter quickly die; but whether this is due to the venom already present in the gland at the time of transplantation, or to venom newly formed and secreted after transplantation, is not determinable from these experiments.

(5) Because of the abnormal conditions under which the gland-tissue lives after transplantation, various abnormalities in its structure are found. The number of the typical granules is in every case diminished. Some changes which we observed are probably due to the absence of an effective excretory duct. Thus the collection of desquamated cells or cell detritus in the ducts, the flattening of certain tubule cells, and the dilation of some ducts may be due to this factor. The frequent shrinking of cells and nuclei and the substitution of the typical tubules by clusters of cells markedly differing in their shape from the normal gland cells are results of the abnormal conditions under which cells live after transplantation.

(6) In the glands from which pieces had been cut no regenerative process could be observed, nor had a noticeable compensatory hypertrophy taken place in the gland of the other side within 4 weeks after the operation.

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D. SOLUBILITY OF THE VENOM GRANULES.

By Henry Fox and Leo Loeb.

Whenever a piece of the fresh poison gland is cut into small particles and the contents of the latter squeezed out into 0.85 per cent (NaCl) salt solution, there results a cloudy emulsion. Microscopic examination shows this to contain innumerable granules of two kinds—spheres and rods. The rods were abundant and were at first supposed to be distinct from the spheres. Nothing like them had been observed in sections of the fixed and stained gland, though in some cases, especially in the formative stages, the intracellular granules were frequently seen to be more or less elongated, but they never showed the clear outlines of the rods as seen in the emulsion. It occurred to us that they might be composite granules; that is, composed of two or several spherical granules arranged in a row. To determine this point they were examined under the oil-immersion objective. In some cases, indeed, the composite nature of the rods appeared fairly evident; in such instances the rod could be seen to be cut across by what appeared to be one or more transverse walls, appearing much like the intercellular partitions in the chain of a rod-shaped bacterium. In other cases the rods showed no clear indications of a composite structure.

The following tests were made to determine the solubilities of the granules. We also wished to examine in a comparative manner the behavior of the rods and spheres. We found that both behaved on the whole alike. This fact renders still more probable the conclusion that the rods and spheres represent the same chemical substance and differ only morphologically.

RECORDS OF TESTS.

A. Solutions of Hydrochloric Acid.

(A^1) 1 c.c. of N/10 HCl added to 1 c.c. of the suspension: Granules dissolved in $1\frac{1}{2}$ minutes. Microscopic examination, 10 minutes later, showed no clear indications of granules. Some minute objects of doubtful nature were observed.

(A^2) 0.1 c.c. N/10 HCl added to 0.5 c.c. suspension: Granules partly dissolved in $1\frac{1}{3}$ minutes. Microscopic examination after 7 minutes showed no certain traces of granules, but free nuclei of blood-corpuscles were present.

(A^3) 0.1 c.c. N/10 HCl added to 0.9 c.c. of the suspension: Liquid remained cloudy, and after an interval of 30 minutes was still slightly opalescent. Microscopic examination, 7 minutes after addition of reagent, showed both granules and rods present, though not in nearly as large a quantity as in a control. A second examination, after 31 minutes, showed no distinct rods or granules. Free nuclei were moderately abundant.

In more dilute solutions of HCl (N/100 to N/250) the granules and rods seem also to become to a great extent dissolved, but here a finely granular precipitate forms in the mixture of solution and acid, and this precipitate makes it difficult to distinguish the preformed from the granules of the precipitate.

B. Tests with Sodium Hydrate.

(B^1) 1 c.c. N/10 NaOH added to 1 c.c. suspension: Granules dissolved after interval of $1\frac{1}{2}$ minutes. Microscopic examination after 10 minutes showed the liquid clear, except for traces of an occasional granule.

(B^2) 0.1 c.c. N/10 NaOH added to 0.5 c.c. suspension: Granules dissolved in 40 seconds. Microscopic examination after 5 minutes showed the liquid clear, with a few nuclei of blood-corpuscles or perhaps also of other cells.

(B^3) 0.1 c.c. N/10 NaOH added to 0.9 c.c. suspension: Granules dissolved in 5 seconds. Microscopic examination, after 9 minutes, showed the liquid clear, with no signs of granules.

(B^4) 0.5 c.c. N/100 NaOH added to 0.5 c.c. suspension: Granules dissolved in 5 seconds. After 27 minutes the liquid was very clear. Microscopic examination after 21 minutes showed the liquid almost clear, with no distinct trace of granules. In alkaline mixtures the fluid becomes clearer than in the acid solutions of corresponding strength. If the alkaline solutions through addition of NaCl are made isotonic with a 0.85 per cent NaCl solution, some of the granules remained somewhat longer preserved than with hypotonic alkaline solutions made up with distilled water.

C. Tests with Solutions of NaCl.

(C^1) 1 c.c. 0.85 per cent NaCl added to 1 c.c. suspension: Granules not dissolved after $1\frac{1}{2}$ minutes. Microscopic examination after 3 minutes showed granules and rods in abundance.

(C^2) 0.1 c.c. 0.85 per cent NaCl added to 0.5 c.c. suspension: Granules not dissolved after 1 minute. Microscopic examination after 8 minutes gave same result as preceding observation. Other similar experiments gave the same results. After 3 hours the fluid had become less cloudy. Microscopic examination at the same time showed that the granules were extremely scarce, only a few distinct ones being seen.

(C^5) 0.5 c.c. 1.5 per cent NaCl added to 0.5 c.c. suspension: Granules not dissolved after 30 seconds. Microscopic examination after $2\frac{1}{2}$ minutes showed the rods and granules well preserved. The same mixture examined after 3 hours showed microscopically only small numbers of granules preserved.

In 0.85 per cent NaCl solution a slow but continuous solution of the granules takes place. They remain better, but not definitely preserved, in a hypertonic NaCl solution, made up by mixing equal quantities of the emulsion of granules and of a 1.7 per cent NaCl solution.

D. Tests with Distilled Water.

(D^1) 1 c.c. H_2O added to 1 c.c. suspension: Granules partly dissolved after $1\frac{1}{2}$ minutes. Microscopic examination after 5 minutes showed number of

granules to have decreased much more than in control. In other experiments with distilled water a large number of the granules disappeared within the first few minutes.

In mixtures of the emulsion and distilled H_2O the granules are not as well preserved as in 0.85 per cent NaCl solution; they are, however, not as rapidly dissolved as in a diluted solution of alkali. Glycerin also causes a disappearance of many granules; at least the granules become less visible a short time after the mixture with the glycerin.

In one experiment (8*n*), 1 c.c. absolute alcohol was added to 0.5 c.c. emulsion. The liquid cleared almost immediately, but showed a slight cloudiness. Microscopic examination, after 3 minutes, showed no granules, only an albuminous precipitate in which a few indistinct granules were intermixed and a few apparent nuclei.

E. Tests with Formalin.

In a mixture of equal amounts of emulsion and of 10 per cent formalin dissolved in 0.85 per cent NaCl solution instead of water, the granules and rods remain distinctly better preserved than in a 10 per cent solution of formalin in distilled H_2O. The limited number of animals which could be used for these experiments, and the fact that each series of experiments meant the sacrifice of an animal, necessitated the limitation in the number of our experiments; yet we believe our results of sufficient interest to warrant publication. We hope that our experiments may be repeated, and, if necessary, in some details corrected in future investigations, but we believe that the following summary will on the whole be found to be correct.

SUMMARY.

(1) The granules are readily soluble in weak solutions of HCl. They dissolve quickly in N/20 and N/50 solutions, but only more slowly dissolve in a N/100 or N/200 solution.

(2) The granules dissolve very quickly in weak solutions of NaOH, even such minute strengths as N/100 and N/200 dissolving them nearly as quickly as the stronger solutions.

(3) The granules are dissolved only very slowly in normal salt solutions. Hypertonic solutions of NaCl preserve the granules better than isotonic solutions.

(4) The granules disappear more rapidly when the normal salt solutions containing them are strongly diluted with distilled water or with glycerin, but solution is not quite as rapid as in the case of HCl and NaOH solutions.

(5) Solutions of formalin dissolved in normal salt solution preserve the granules better than 10 per cent formalin prepared with distilled water.

(6) The rods and spheres react in a similar manner toward the various reagents.

Under normal conditions the granules probably are dissolved before the venom enters the gland-ducts. At least we do not find granules in the contents

of the venom gland-ducts. If we collect venom in the manner usually employed in our work, we find the fluid collected from the mouth of the animal to contain cells of an epithelial character and leucocytes. Many of these cells contain granules, and through disintegration of cells nuclei get into the surrounding fluid. In one case we saw only leucocytes in a drop which we collected, and the fluid was here free from granules. The large majority of granules found in the venom collected from the mouth are probably not derived from the venom gland, but from epithelial cells of the mouth and perhaps of some neighboring glands.

II.

GENERAL PROPERTIES AND ACTIONS OF THE VENOM OF HELODERMA, AND EXPERIMENTS IN IMMUNIZATION.

By Elizabeth Cooke and Leo Loeb.

(With Some Additional Experiments by Moyer S. Fleisher.)

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REVIEW OF THE LITERATURE.

The Gila monster is a reptile found in the southwestern portion of the United States and also in Mexico. The *Heloderma suspectum* is usually found in the United States; in Mexico the *Heloderma horridum* is more common. The bite of either species is supposed to have a distinctly poisonous effect; however, the general evidence in the literature regarding the toxic effects of the venom of these animals is distinctly contradictory. The physiological action of the venom has been studied by several observers: Sumichrast (Note on the habits of some Mexican reptiles, Ann. and Mag. Nat. Hist., vol. 13 (3), p. 497, 1864); Boulenger (Proc. Zool. Soc., London, 1882, p. 631); A. Dugés (Cinquantenaire de la Soc. de Biologie, Volume jubélaire, publié par la Société, p. 134, Paris, 1899); Gorman (Bull. Essex Institute, Salem, Mass., vol. 22, p. 60, 1890); and Boncourt (Compt. rend. de l'Acad. des Sciences, vol. 80, p. 676, 1875). Boncourt caused animals of different species to be bitten by helodermas and noted that the bite was fatal to smaller animals, such as frogs, chickens, rabbits, and guinea-pigs, while dogs and cats developed serious symtoms, from which, however, they usually recovered. Besides these observations, there have been reported instances of more or less serious disturbances arising from accidental injuries to human beings, but in regard to these the evidence is very conflicting and appears to be scarcely trustworthy.

Mitchell and Reichert (Medical News, vol. 42, p. 209, 1883; Science, vol. 1, p. 372, 1883; Amer. Nat., vol. 17, 800, 1883) were the first to collect the venom and to study in detail the symptoms produced by injecting it into animals in known quantities. They caused the helodermas to bite upon a saucer edge and with the secretion thus obtained they made experiments upon frogs, rabbits, and pigeons. Since that time several other investigators have collected the venom and have investigated its properties.

Santesson (C. G. Santesson, Ueber das Gift von *Heloderma suspectum* Cope, einer giftigen Eidechse, Nordiskt Medicinskt Arkiv. Testband tillegnadt Axel Key, Nr. 5, 1897) obtained the poisonous secretion by causing animals to chew upon small sponges, which he afterwards washed out with physiological salt solution. The venom thus obtained he studied with reference to its chemical constitution and with it also made a number of experiments on frogs, rabbits, and mice.

Van Denburgh (Trans. Am. Phil. Soc., vol. 19, 1898, p. 199) and Van Denburgh and Wight (Am. Jour. Phys., vol. 4, 1900, p. 209) collected the secretion either by causing the helodermas to chew filter paper or to bite on rubber. They made observations on the physiological effects of the venom upon dogs, cats, and frogs, and also a few experiments to test its effect upon the red blood-corpuscles.

We have studied the venom of both species in regard to its physiological action, in its influence in causing hemolysis of red blood-cells, and in several other ways.

METHOD OF COLLECTING THE VENOM AND FACTORS WHICH INFLUENCE THE AMOUNT OF SECRETION.

A number of small ducts lead from the poison gland into a groove between the lower lip and the teeth of the lower jaw. When the animal bites the secretion gushes into this groove. The ducts open near the bases of the small pointed teeth; consequently the venom is ejected in the neighborhood of the several wounds made by the teeth, the grooves present on the outer side of the teeth carrying the venom into the wound; there is, however, no means by which the *Heloderma* can inject venom directly into an enemy, as does the *Crotalus*.

In order to collect the venom we caused the animals to bite upon a piece of soft rubber and with a capillary pipette drew off the secretion from the lower side of the rubber. This method of collecting has the double advantage of yielding a maximum of the secretion and also secretion from the lower jaw only. An animal in good condition will usually chew the rubber for several minutes and each time the jaws close there is a gush of venom into the groove, but the length of time which the chewing continues, as well as the amount of secretion which flows out at each bite, is subject to great variations. Chief among the factors which influence these variations are:

(1) The condition of the teeth. The teeth are easily and frequently injured, and animals without teeth spit out the rubber as soon as it is placed in the mouth. Probably the reason that animals with good teeth do not spit out the rubber is that the teeth become caught in the rubber, which thus can not be pushed out by the tongue.

(2) The time which has elapsed since the previous collection. If the secretion is taken every day the amount falls off rapidly, and even if the animals chew well the flow is quickly exhausted and may at times be absent altogether.

(3) The nutritive condition of the animal. We found that animals which had not eaten food for some time yielded little or no secretion, but that soon after they began to take food again the secretion became abundant.

Besides these factors it seems that the temperature and the length of time since the last feeding also play a rôle, but the evidence on these heads is not sufficient to enable us to draw definite conclusions. It is also probable that when animals are kept for a considerable length of time in captivity they gradually lose the power of secreting venom.

INFLUENCE OF PILOCARPINE ON THE SECRETION OF VENOM.

In order to obtain greater quantities of venom we injected subcutaneously 0.1 grain of pilocarpine into a heloderma from which all secretion obtainable by the usual method had been collected. As a rule, in from 1 to 3 minutes after the injection the flow began again and lasted now 20 to 30 minutes, reaching its maximum at 10 to 15 minutes after injection of pilocarpine. The flow induced by pilocarpine not only lasts longer, but is more abundant than the ordinary flow and frequently reaches three or four times the amount obtained without pilocarpine. However, the influence of pilocarpine can not be maintained permanently. By its use venom may be obtained from an animal that has just previously yielded none, but if such an animal be injected with pilocarpine on the succeeding day no secretion will be obtained in consequence. We found that in animals yielding venom freely the first injection of pilocarpine caused increased secretion; the second injection (on the succeeding day) caused a secretion where none was previously to be obtained; the third injection was without result. In those animals which secreted venom freely the first injection of pilocarpine caused an increase in the secretion of venom; when the second injection of pilocarpine was given on the day following the first injection, the venom glands were again stimulated to secretion of venom, although before the injection of pilocarpine it had been impossible at this time to collect any venom; when a third injection of pilocarpine was given to such an animal on the day following the second injection, no venom was secreted.* This failure of the venom glands to respond to the third injection of pilocarpine is probably due to exhaustion of the secreting-cells, an exhaustion from which the cells are unable to recover in so short a period of time as 24 hours.† As a rule, the first injection causes also discharge of urine and feces, whereas succeeding injections do not have this effect. The variations in the reaction of the *Heloderma* to succeeding injections of venom are perhaps partly to be explained by the fact that the animals were fed but once or twice a week. After the first two doses of pilocarpine have set up a genuine secretion of the venom, a new formation of the venom takes place only very insufficiently, and a third dose of pilocarpine is therefore without much effect. After a third injection the gland-cells still secrete the very small amount of venom stored up within the cells. Whether the pilocarpine, besides causing a real secretion of the venom, also causes a discharge of venom already secreted, we can not state.

Twenty-one helodermas in all were investigated with reference to the influence of pilocarpine upon the amount of secretion. In every case the first dose brought about increase of secretion. The second dose also produced increased flow when the animal had been previously yielding well, and only at the third dose was there no increase. Three animals were subjected to more than three successive injections and all of these died. Sixty animals—most

*In one case an animal which had responded to a first injection of venom did not give an increased amount of venom under the influence of a second injection of pilocarpine given 40 hours later.

†These observations on the functional effect of pilocarpine are in good agreement with the observations concerning the effect of pilocarpine on the morphology of the venom gland recorded in the paper by Fox and Loeb.

of them rats and guinea-pigs—were used in testing the comparative strength of venom obtained with and without pilocarpine. The venom collected after injection of pilocarpine was at least as active as the venom obtained without pilocarpine.

Influence of injection of Pilocarpine on secretion of venom in Heloderma.

Experiment 1 (May 14):

10h15m Inserted rubber in mouth; rapid flow of saliva.
10 25 Diminishing flow.
10 30 Flow ceased; 2 c.c. collected.
10 30 Injected 0.1 grain pilocarpine in 1 c.c. physiological salt solution; animal shows immediate weakness; ceases to bite; no flow of saliva.
10 40 Flow of saliva beginning.
11 00 Good flow; mucus increasing; animal again bites well.
11 15 Flow finished; about 2 c.c. collected.

Experiment 2 (May 20):

Collected nearly 1 c.c. of saliva from large heloderma during 20 minutes; injected 0.1 grain pilocarpine in 1 c.c. physiological salt solution; collected nearly 1 c.c. during next 20 minutes; urine and feces voided; no marked signs of weakness.

Experiment 3 (May 20):

Collected nearly 1 c.c. saliva from large heloderma in 30 minutes; injected 0.1 grain pilocarpine; flow began in about 5 minutes; collected in 15 minutes about 2 c.c.; became viscid and flow diminished; collected in next 15 minutes about 1 c.c.

Experiment 4 (May 31):

Collected during 30 minutes about 0.7 c.c. saliva from one large heloderma; secretion ended, injected 0.1 grain pilocarpine; 5 minutes later secretion began again; during 30 minutes collected 1.5 c.c.

Experiment 6a (June 12):

Collected venom from 8 helodermas; got from 8 only about 0.4 c.c.; gave 2.1 grains each of pilocarpine; collected about 1.4 c.c.

Experiment 6b (June 13):

Injected same helodermas with pilocarpine; one gave no venom before or after injection; the other was not tried before, but gave after injection only about 0.2 c.c.; all helodermas not tried on the 12th were tried and gave practically no saliva; to be fed to-night.

Experiment 6c (June 14):

Two helodermas injected with pilocarpine on third successive day; no secretion.

Experiment 6, cont. (June 17):

One heloderma that received pilocarpine on three successive days (June 12–14) dead.

Experiment 7 (June 18):

Took venom from all helodermas (18); 4 that have been in the laboratory and have eaten two successive nights gave most of it; 2 were injected with pilocarpine; 1 which had given none before gave scarcely any, the other (one that had been in the laboratory) gave the usual increased secretion; collected altogether only 2.2 c.c.

Comparative toxic effect of venom obtained with and without injection of pilocarpine.

Guinea-pig 29, 100 g.
May 20, 4h 12m injected 0.066 c.c. venom of heloderma No. 1 in 1 c.c. physiological salt solution; secretion obtained before injection of pilocarpine. *5 o'clock* weak; breathing slow, not difficult. *6 o'clock* dead.

Guinea-pig 28, 500 g.
May 20, 4h 10m injected 0.066 c.c. venom from heloderma No. 1 obtained after injection of pilocarpine. *5 o'clock* paralyzed; breathing slow and quiet. At *6 o'clock* dead.

Guinea-pig 31, 500 g.
May 20, 4h 16m injected 0.066 c.c. venom from heloderma No. 2 obtained before injection of pilocarpine. *5 o'clock* animal affected, sits upright, able to right itself when put on side. *6 o'clock* symptoms grave; not likely to recover. May 21 found dead.

Guinea-pig 32, 480 g.
May 20, 4h 14m injected 0.66 c.c. venom from heloderma No. 2 obtained after injection of pilocarpine. First portion before secretion of mucus was abundant. *5 o'clock* paralyzed; breathing quick, not difficult. 5h 20m dead.

Guinea-pig 33, 480 g.

MAY 20, 4^h 17^m injected 0.066 c.c. venom from heloderma No. 2 obtained after injection of pilocarpine; second portion after secretion had become very slimy. *5 o'clock* paralyzed; breathing quick, not labored; occasional convulsive movements of legs. 5^h 20^m breathing irregular, slow; jerking movements of head and legs. *6 o'clock* dead.

Rat 6, 120 g.

MAY 24, *6 p. m.* injected 0.1 c.c. venom from heloderma No. 1 obtained before injecting pilocarpine. 6^h 25^m appears weak, unable to move easily. *7 o'clock* somewhat better. MAY 25 found dead.

Rat 7, 120 g.

MAY 24, 5^h 56^m injected 0.1 c.c. venom from heloderma No. 1 obtained after injecting pilocarpine. 6^h 30^m very weak. *7 o'clock* labored breathing; reflexes absent. 7^h 10^m dead.

Venom before injection.

Rat 58, 100 g.

JUNE 7, 5^h 35^m injected 0.1 c.c. venom. 6^h 30^m dying. *8 o'clock* dead.

Guinea-pig 48, 420 g.

MAY 31, 12^h 03^m injected 0.1 c.c. venom. 1^h 30^m sits down; breathing rapid. *2 o'clock* inclined to fall on one side; able to right itself. 2^h 30^m breathing difficult, jerky. 3^h 25^m occasional violent jerks. *4 o'clock* dead.

Rat 15, 61 g.

MAY 31, 12^h 14^m injected 0.1 c.c. venom. 12^h 30^m lying on side; breathing rapid. 12^h 40^m lying on side; breathing slow. 12^h 45^m reflexes absent. 12^h 50^m dead.

Venom after injection.

Rat 59, 100 g.

JUNE 7, 5^h 40^m injected 0.1 c.c. venom. 6^h 30^m dead.

Guinea-pig 47, 600 g.

MAY 31, *12 o'clock* injected 0.1 c.c. venom. 12^h 30^m lying down. 12^h 40^m lying down; breathing rapid. 1^h 30^m lies on side. *2 o'clock* breathing very forced; right legs paralyzed. 2^h 30^m breathing difficult; slower. 2^h 45^m tetanic convulsions when stimulated. 2^h 50^m dying; convulsions when stimulated. 3^h 10^m dead.

Rat 14, 98 g.

MAY 31, 12^h 10^m injected 0.1 c.c. venom. 12^h 30^m lying on side; breathing rapid. 12^h 40^m lying on side; breathing slow. 12^h 45^m reflexes present. 12^h 50^m reflexes present. *1 o'clock* reflexes absent. 1^h 10^m better; able to move. *2 o'clock* lies on side; apparently dying; reflexes present. 2^h 20^m dead.

EFFECT OF HEAT ON VENOM.

Santesson (Nordickt Medicinskt Arkiv. Testband Ullegnadigt Axel Key, Nov. 5, 1897) showed in a few experiments that boiling the venom for at least 10 minutes does not diminish its toxicity. We have found, however, that boiled and unboiled portions of the same venom were equally toxic only when the precipitate was not filtered off from the former. Some of the venom was evidently carried down with the precipitate, but if precautions were taken so that the precipitate was fine enough to be injected there was no appreciable difference in the effect of the boiled and unboiled portions. In some cases where the filtrate from the boiled venom showed a lower toxicity than the original sample we found that the precipitate, mixed with physiological salt solution and injected into an animal, was toxic. We found the same to be true concerning the precipitate from unboiled venom, *i.e.*, the precipitate formed by diluting venom with ten times its volume of physiological salt solution gives, when injected into an animal, all the effects produced by injecting the precipitate from boiled venom.

Since it was possible to heat the fresh venom to 100° without decreasing its toxicity we were able to sterilize the venom before injecting it into animals. This precaution was manifestly necessary, since it was found that the fresh

venom contained large numbers of bacteria, some of which proved to be extremely virulent for guinea-pigs.* In making use of sublethal doses and of doses which kill only after a considerable interval, it was of course necessary to eliminate the influence of micro-organisms. Almost all our experiments have been made with venom sterilized either by boiling for 10 minutes or by being kept for an hour at a temperature of 60°. The advantage of the latter method is that the resulting precipitate is a much finer one and passes easily through a fine hypodermic-syringe needle.

A large number of tests were made comparing the toxicity of heated and unheated venom. The toxicity of the venom was tested in guinea-pigs, rats, rabbits, mice, and tadpoles. The venom used in these experiments had been heated either to 60° C. for an hour, 100° C. for 10 minutes or 30 minutes, or to 120° C. in the autoclave for 15 minutes. The injection of any of the heated venoms led to the death of the animals almost as quickly as the injection of unheated venom.

Thus it was apparent that heat of such degrees as we have used in these experiments does not injure the toxicity of the fresh venom to any marked degree. However, it was noted occasionally that when animals were injected with small quantities of the heated venom (that is, quantities which were but very little larger than the lethal dose), such animals lived longer after the injection than animals injected with corresponding quantities of the unheated venom. Therefore it appears that the heating exerts some slight injurious effect on the venom. This slight decrease in the toxicity after heating may perhaps be due to an inclusion of some venom in the coagulum. It will presumably require some time for the included venom to be liberated; the action of the venom is, therefore, distributed over a longer period of time and is consequently less acute.

With dried venom we tested the influence of exposure to temperature of 80° C. for 1 hour, 100° C. for 1 hour, 120° C. (autoclave) for both 1 and 2 hours. In these experiments only mice were used. It was found that the heating of the dissolved dry venom did not diminish its toxicity, although in a few cases it was observed that animals injected with the smaller doses of the heated venom did not die quite as soon as animals injected with the same quantities of unheated venom. Santesson records the fact that the dried venom would not endure heating to 110° C., while the fresh venom would. The reason for this difference between Santesson's observations and ours is not clear.

The action of heat upon various snake venoms differs; thus, the venom of Colubridæ (*Naja, Bungarus, Holocephalus,* and *Pseudechis*) and of Hydrophinæ may be heated to 100° C. and even boiled for a short time without injury; heating for a long time to 100° C. or heating to 120° C. diminishes or destroys the toxicity. The venom of Viperidæ (*Lachesis, Crotalus, Vipera*) is injured by being heated to 70° C. and is destroyed at 80° to 85° C.

Unlike the heloderma venom, the venom of the Colubridæ gives a precipitate which appears after heating to 72° C. and which is not toxic.

*Bacteria belonging to the group of the colon were especially virulent, as found by Dr. D. Rivas.

The heloderma venom is rather more resistant to the destructive action of heat than any of the above-named snake venoms, since even though heated to 120° C. the toxic action is weakened but not destroyed.

Below we give a few experiments showing the influence of heating on the toxicity of the venom.

Boiled Venom (filtered).

Rat 12, 110 g.

MAY 28, 3^h 22^m injected 0.1 c.c. venom. *4 o'clock* very weak, appears dying. 4^h 30^m somewhat better. *5 o'clock* a little better, able to slowly right itself if placed on back. 5^h 30^m still living, condition very bad. 6^h 25^m dying. Found dead.

Guinea-pig 41, 400 g.

MAY 27, 4^h 23^m injected 0.1 c.c. venom. 6^h 35^m drowsy, occasional starts, able to right itself when placed on side. *7 o'clock* occasional convulsive jerks, position drooping. 8^h 30^m occasional violent jerks, position abnormal. MAY 28, *9 a. m.* much improved. MAY 29 recovered.

Unboiled Venom.

Rat 13, 110 g.

MAY 28, 3^h 35^m injected 0.1 c.c. venom. *4 o'clock* slightly worse than rat 12. 4^h 30^m improving, still worse than rat 12. *5 o'clock* dying. 5^h 30^m dead.

Guinea-pig 41, 340 g.

MAY 27, 4^h 30^m injected 0.1 c.c. venom. 6^h 30^m forced breathing; lies on side; very weak. *7 o'clock* dying. 7^h 05^m dead.

Precipitate from Boiled Venom used on May 28.

Rat 14, 100 g.

MAY 29, 3^h 55^m injected. *6 o'clock* rather sick. MAY 30, *9 a. m.* found dead.

Boiled Venom (not filtered).

Guinea-pig 45, 800 g.

MAY 29, 3^h 47^m injected 0.2 c.c. venom. 5^h 30^m lying down, breathing labored. 5^h 45^m dying, occasional jerking movements. 5^h 50^m convulsive jerking, extremely irritable. *6 o'clock* dead.

Unboiled Venom.

Guinea-pig 46, 700 g.

MAY 29, 3^h 55^m injected 0.2 c.c. venom. 5^h 30^m bad condition, slightly better than guinea-pig 45. *6 o'clock* dying. 6^h 05^m dead.

Venom Dried below 70°.

Mouse 41.

NOV. 13, 2^h 20^m injected 0.2 mg. intraperitoneally. 4^h 25^m dead.

Same Dried Venom Heated 1 hour to 116°.

Mouse 45.

NOV. 13, 3^h 15^m injected 0.2 mg. intraperitoneally. 5^h 30^m dead.

EFFECT OF STANDING IN SOLUTION ON TOXICITY OF VENOM.

A 10 per cent solution of venom in physiological salt solution kept sterile and allowed to stand for 9 days in the laboratory showed no decrease in virulence; but venom sterilized and merely covered became, under the same conditions, less toxic. This doubtless is due to the action of bacteria entering it from the air. To guard against possible complications due to the presence of bacteria in venom which had been allowed to stand, it was resterilized before being used for inoculations.

June 8 a quantity of venom was sterilized and its strength tested on a mouse and a rat. It was divided into two portions, one of which was kept sterile and the other simply covered, so as to prevent the entrance of dust. Both portions were left for 9 days in the laboratory at room temperature and in the light. On June 17 they were heated to 60° C. for 45 minutes and used in the following experiments:

Sterile Venom.

Rat 96, 120 g.

JUNE 17, $12^h\ 05^m$ injected intraperitoneally 0.1 c.c. venom. $1^h\ 30^m$ found dead.

Mouse 17.

JUNE 17, $12^h\ 28^m$ injected intraperitoneally 0.01 c.c. venom. $1^h\ 30^m$ found dead.

Not Sterile Venom.

Rat 97, 120 g.

JUNE 17, $12^h\ 08^m$ injected intraperitoneally 0.1 c.c. venom. $1^h\ 30^m$ living; sick. $2^h\ 45^m$ dead.

Mouse 18.

JUNE 17, $12^h\ 31^m$ injected intraperitoneally 0.01 c.c. venom. $1^h\ 30^m$ living; very sick. *2 o'clock* dying; killed.

Other experiments were also carried out with venom which had been treated in a similar manner, that is, sterilized and kept at room temperature. This venom was tested 2, 7, and 21 days after sterilization, and it was found that while it had lost part of its toxicity it had lost far less than the unsterilized venom which was kept on ice. Thus, on the day the venom was sterilized, 0.005 c.c. of unheated venom killed a mouse in from 25 minutes to 4 hours 30 minutes; 2 days later, 0.005 c.c. of sterilized venom killed a mouse in 8 hours; 7 days later, 0.01 c.c. of unsterilized venom killed a mouse in 24 hours, while a similar quantity of sterilized venom killed in 6 hours; 21 days after the sterilization, 0.03 c.c. of the sterilized venom killed a mouse in 40 minutes, while the same quantity of the unsterilized venom killed in 24 hours.

In other experiments the venom, after being sterilized, was placed in the thermostat and kept at a temperature of 37.5° C. instead of being kept at room temperature. In these experiments also it was noted that the sterilized venom kept better than the unsterilized venom. However, in all cases, the toxicity of the venom when it had been dissolved diminished slightly after some time, even though it had been sterilized.

Furthermore, we tested the power of thymol to prevent the deleterious effect on venom of bacteria and of standing in solution. We found that at periods 2, 7, and 21 or 23 days after mixing, unsterilized venom to which thymol had been added had lost its toxicity in much the same degree as unsterilized venom to which no thymol had been added. Sterilized venom, to which thymol had been added, acted in much the same manner as the simple sterilized venom; that is, became very slightly less toxic after several days.

Thus we note that unsterilized venom in solution loses its toxicity fairly rapidly because of bacterial action. Sterilized venom in solution loses in toxicity far more slowly, and here the loss of toxicity is probably due to the gradual decomposition of the venom when kept in solution. The addition of thymol does not appear to prevent the destruction of the venom, which, in spite of the thymol, loses its toxicity as rapidly as unsterilized venom.

EFFECT OF DIALYZING.

The venom passes through parchment paper rather slowly. In our experiments venom was diluted to ten times its original volume with physiological salt solution, sterilized and put to dialyze against twice its volume of sterile salt solution. The whole apparatus (made as sterile as possible) was kept directly on ice. In $4\frac{1}{2}$ days—the longest period that we tried—dialysis of the

toxic substances was not complete. The results obtained by injecting into animals, at different intervals, material from inside and outside the dialyzer were as follows: At the end of 1 day the solution outside the dialyzer had acquired scarcely any of the toxic substance and the solution inside showed scarcely any decrease in toxicity; at the end of 2 days the symptoms produced by material from outside the dialyzer were marked but not grave, while death followed the injection of a similar quantity of the solution inside the dialyzer; at the end of 3 days the symptoms produced by the injection of fluid from outside the dialyzer were grave, but less serious than those produced by material from inside the dialyzer; at the end of 4 days the symptoms produced by material from outside the dialyzer were grave and with large doses sometimes fatal, but they were still less severe than the symptoms produced by similar quantities of the material within the dialyzer. Throughout there was noted a gradual decrease of the toxicity of the solution inside the dialyzer. Where large quantities of the solution were injected, control animals were injected with equal volumes of sterile salt solution. All the solutions were sterilized before injecting. In these experiments mice and rats were used to test the toxicity of the solutions. In this respect the heloderma venom behaves in a manner similar to the venom of the Colubridæ, which passes slowly through vegetable membranes. The gradual decrease in toxicity of the solution within the dialyzer and the corresponding increase in toxicity of the solution outside the dialyzer is apparent in the following protocols:

Venom Dialyzing 24 hours.

Outside Dialyzer.

Rat 104, 100 g.

JUNE 25, *4 o'clock* injected subcutaneously 1 c.c. of solution. $4^h\ 45^m$ slightly affected. JUNE 26 recovered.

Mouse 26.

JUNE 25, $4^h\ 02^m$ injected subcutaneously 0.2 c.c. of solution. $4^h\ 45^m$ respiration rapid; otherwise well. JUNE 26 recovered.

Inside Dialyzer.

Rat 105, 110 g.

JUNE 25, $4^h\ 15^m$ injected subcutaneously 1 c.c. of solution. $4^h\ 45^m$ very gravely affected. $4^h\ 55^m$ dead.

Mouse 27.

JUNE 25, $4^h\ 16^m$ injected subcutaneously 0.2 c.c. of solution. $4^h\ 45^m$ lying down; breathing forced. *5 o'clock* dead.

Venom Dialyzing 44 hours.

Outside Dialyzer.

Rat 24, 100 g.

JUNE 5, $12^h\ 15^m$ injected intraperitoneally 4 c.c. of solution; appeared somewhat drowsy all day, but recovered.

Rat 26, 100 g.

JUNE 5, $12^h\ 17^m$ injected intraperitoneally 2 c.c. of solution; drowsy; recovered.

Rat 28, 120 g.

JUNE 5, $12^h\ 18^m$ injected intraperitoneally 1 c.c. of solution; slightly drowsy; recovered.

Inside Dialyzer.

Rat 25, 100 g.

JUNE 5, $12^h\ 20^m$ injected intraperitoneally 4 c.c. of solution. $12^h\ 30^m$ lying down; very much affected. $12^h\ 40^m$ dead.

Rat 27, 100 g.

JUNE 5, $12^h\ 22^m$ injected intraperitoneally 2 c.c. of solution. $12^h\ 40^m$ breathing difficult, lies on side. $12^h\ 45^m$ breathing very difficult, legs paralyzed. $12^h\ 47^m$ reflexes absent. *1 o'clock* dead.

Rat 29, 120 g.

JUNE 5, $12^h\ 25^m$ injected intraperitoneally 1 c.c. of solution. $12^h\ 45^m$ lies on side. *1 o'clock* dying. $1^h\ 30^m$ dead.

Venom Dialyzing 49 hours.

Outside Dialyzer.

Rat 109, 105 g.

JUNE 26, 5^h 10^m injected subcutaneously 1 c.c. of solution. *6 o'clock* slightly affected. JUNE 27 recovered.

Mouse 28.

JUNE 26, 5^h 11^m injected subcutaneously 0.2 c.c. of solution. *6 o'clock* affected, but much less than mouse 29. JUNE 27 recovered.

Inside Dialyzer.

Rat 110, 110 g.

JUNE 26, 5^h 12^m injected subcutaneously 1 c.c. of solution. *6 o'clock* lying down, very sick. JUNE 27 dead.

Mouse 29.

JUNE 26, 5^h 13^m injected subcutaneously 0.2 c.c. of solution. *6 o'clock* nearly dead. JUNE 27 dead.

Venom Dialyzing 67 hours.

Rat 112, 100 g.

JUNE 27, 11^h 30^m injected subcutaneously 1 c.c. of solution. *12 o'clock* lying down. 12^h 30^m lying down. *1 o'clock* lying down. *2 o'clock* improving. JUNE 28 recovered.

Mouse 30.

JUNE 27, 11^h 30^m injected subcutaneously 0.2 c.c. of solution. *12 o'clock* lying down. *1 o'clock* better. JUNE 22 recovered.

Rat 113, 110 g.

JUNE 27, 11^h 32^m injected subcutaneously 1 c.c. of solution. *12 o'clock* lying down; about same as rat 112. 12^h 30^m lying down, not much worse than rat 112. *1 o'clock* worse than rat 112. *2 o'clock* dead.

Mouse 31.

JUNE 27, 11^h 33^m injected subcutaneously 0.2 c.c. of solution. *12 o'clock* lying down. *1 o'clock* dead.

Venom Dialyzing 112 hours.

Rat 84, 85 g.

JUNE –, 10^h 52^m injected subcutaneously 2 c.c. of solution. *2 o'clock* sick, but may recover. JUNE 14 well.

Rat 88, 120 g.

JUNE –, 11^h 20^m injected subcutaneously 1 c.c. of solution. 1^h 20^m seriously affected. JUNE 14 recovered.

Rat 85, 85 g.

JUNE –, 10^h 56^m injected subcutaneously 2 c.c. of solution. *2 o'clock* dead.

Rat 89, 130 g.

JUNE –, 11^h 22^m injected subcutaneously 1 c.c. of solution. 1^h 20^m dead.

Mouse 13.

JUNE –, 12^h 44^m injected subcutaneously 0.2 c.c. of solution. 1^h 50^m seriously affected. 2^h 35^m dead.

Mouse 14.

JUNE –, 12^h 45^m injected subcutaneously 0.2 c.c. of solution. 2^h 20^m dead.

EFFECT OF FILTRATION.

The venom of *Heloderma* passes unchanged through a Chamberland filter. Two similar portions of venom, one filtered, the other unfiltered, produce, when injected into animals, identical results, that is to say, they bring about similar symptoms and kill in approximately equal times. We found in ten rats which we injected that exactly similar results were produced by equal doses of filtered and unfiltered venom.

Again, it may be noted that the venom of *Heloderma* acts in a manner similar to the venoms of the Colubridæ, which pass through a Chamberland filter, while that of the Viperidæ does not.

Filtered Venom.

Rat 31, 140 g.

JUNE 5, 4^h 24^m injected intraperitoneally 0.1 c.c. venom. 6^h 30^m drowsy. JUNE 6, *9 a. m.*, found dead.

Rat 33, 120 g.
JUNE 5, 4h 22m injected 0.05 c.c. venom intraperitoneally. JUNE 6, well, no symptoms.
Rat 54, 190 g.
JUNE 7, 5h 27m injected intraperitoneally 0.2 c.c. venom. 6h 30m dying. 8 o'clock dead.
Rat 56, 120 g.
JUNE –, 5h 28m injected subcutaneously 0.1 c.c. venom. 6h 30m slightly sick. 8 o'clock dead.

Unfiltered Venom.

Rat 30, 140 g.
JUNE 5, 4h 03m injected intraperitoneally 0.1 c.c. venom. 6h 30m drowsy. JUNE 6, 9 a. m., found dead.
Rat 32, 120 g.
JUNE 5, 4h 01m injected 0.05 c.c. venom intraperitoneally. JUNE 6, well, no symptoms.
Rat 55, 190 g.
JUNE 7, 5h 25m injected intraperitoneally 0.2 c.c. venom. 6h 30m dying. 8 o'clock dead.
Rat 57, 130 g.
JUNE –, 5h 30m injected subcutaneously 0.1 c.c. venom. 6h 30m slightly sick. 8 o'clock dead.

INFLUENCE OF THE ADDITION OF ACID TO VENOM SOLUTION.

In connection with certain experiments which we performed it was of interest to determine whether the addition of acid to a solution of venom influenced the toxicity of this substance. It has been shown that the venom, in a solution of either fresh or previously dried venom, is neutral in reaction. We added to 5 c.c. of a 10 per cent (fresh) venom solution 2 c.c. of a N 10 HCl solution. No precipitate was produced. After allowing this mixture to stand for some time we added 2 c.c. of N/10 NaOH solution in order to neutralize the previously added acid.

Various quantities of this venom solution were injected into mice, and at the same time other mice were injected with similar quantities of the pure, equally diluted venom solution. In every case the mice injected with the neutralized acid solution of venom died in approximately the same length of time as the animals injected with the pure venom. The addition of acid to heloderma venom does not, therefore, change the toxicity of the venom.

Influence of addition of acid to venom solution.

Quantity of venom injected.	Time between injection and death.	
	Controls.	Acidified venom.
c.c.	h. m.	h. m.
0.011	3 ..	5 ..
.022	3 ..	3 ..
.055	1 ..	1 20
.055	2 15	3 ..
.027	1 ..	2 ..

EFFECT OF ROENTGEN RAYS ON SECRETION OF VENOM GLAND.

In order to test the influence of exposure to Roentgen rays on the properties or secretion of the venom we exposed several of the helodermas to the Roentgen-ray illumination. Finding that single exposures produced no appreciable result, we subjected two helodermas to a period of treatment extending over a period of about 3 weeks. The lower jaws of the animals, with the venom

glands, were exposed every other day for 10 minutes. At the end of the treatment we found that venom from these individuals, instead of having diminished in virulence, was somewhat more active than venom taken from other animals which had not been exposed to the Roentgen ray, a fact that we attributed to the influence of the long period of rest enjoyed by the glands of the Roentgenized helodermas. Six rats injected with the first venom taken from these animals showed that its toxicity had undergone no change that could be attributed to the Roentgen-ray treatment.

EFFECTS OF VENOM UPON ANIMALS.

When a warm-blooded animal receives a lethal dose of heloderma venom the first conspicuous effect is a disturbance of respiration. The breathing becomes quickened and the respirations are forced. After a time the respiratory rate diminishes and the respirations grow shallow; finally they occur only at long intervals, until after a period of respiratory spasm they cease altogether. Meanwhile, other symptoms appear. The animal shows weakness and falls down, and frequently, though not as a rule, has acute attacks of convulsions. Reflex irritability becomes increased so that a slight sensory stimulus is able to inaugurate an exaggerated response which resembles the response obtained in an animal poisoned by strychnine. The hind legs hang as if paralyzed and later the forelegs also appear paralyzed. If the skin of the leg be pinched, however, a response is elicited, and this response may still be obtained very shortly before the last respiration. The corneal reflex may be obtained even later than the sensory skin-reflex.

It was noted that when mice were injected subcutaneously with lethal quantities of venom, one hind leg became completely paralyzed and spastic at a time when complete paralysis was not present in the other hind leg. This unilateral spastic and paralytic effect was not due to the venom being injected into or near this leg, since the fluid was usually injected under the skin of the thorax. Not only was the one leg spastic and paralyzed, but sensation was almost completely lost in this leg. Pinching the skin or pricking with a pin produced no response; occasionally, however, pressure (pinching the entire leg) would lead to a slight muscular response of the other leg. These symptoms are general for all the mammals which we have used, viz, dog, cat, guinea-pig, rabbit, rat, and mouse; but some of them appear more conspicuously in certain species than in others. For instance, the apparent paralysis appeared most marked in rats and mice and the strychnine-like response to stimulus was most evident in guinea-pigs. The respiratory disturbances were substantially the same in all animals, and it is to the interference with respiration that we must attribute the immediate cause of death in the warm-blooded animal.

Besides the abnormal symptoms common to all, certain species of animals show other disturbances. In dogs and cats injection of venom causes vomiting and discharge of urine and feces. It also causes a very copious flow of saliva and of tears. In cats a noticeable symptom is loss of voice. The animal makes continuous crying movements, but without the production of

sound. In rabbits and guinea-pigs there occurs occasionally a conspicuous flow of tears, and guinea-pigs develop an extreme rigidity of the abdominal walls. In order to see whether this symptom might be due to the operation, we injected control guinea-pigs with salt solution and found that the abdominal walls remained relaxed.

The heart continues to beat after the respiration has ceased, sometimes for a considerable time—10 to 30 minutes in the warm-blooded animals and many hours in frogs. The auricles beat after the ventricle has stopped. Occasionally the cardiac rhythm is disturbed, the auricles beating oftener than the ventricle. As a rule the heart stops in diastole, but may at times stop in systole.

Santesson records a curare-like effect of the poison, which was not manifested in our experiments. We found that in frogs which had died several hours previously as a result of the injection of venom, mechanical stimulation of the sciatic nerve would cause twitching of the leg muscles, and the same effect was observed in mice immediately after death.

Mitchell and Reichert believed the venom to be a heart poison. In the few experiments that they made they used very large doses and brought about death very suddenly. Probably by this means they caused a reflex stopping of the heart, but that death is not due to a direct effect upon the heart we saw in very many cases where mammalian hearts continued to beat for 30 minutes and frogs' hearts for several hours after death.

Denburgh and Wight (Am. Journ. Phys., 1900, 4, p. 209) had previously reached a conclusion identical with ours; they also had decided that the immediate cause of death was in respiratory failure. The observations of Denburgh and Wight upon the physiological effects of heloderma venom are in substantial agreement with our own. We have injected more than 360 warm-blooded animals with heloderma venom, and although these have not all received continuous detailed inspection, nevertheless many have been kept under close observation. From the history of these, we have selected the following typical records:

Rabbit 2, 500 g.

May 30, $9^h\ 20^m$ injected intraperitoneally 0.15 c.c. sterile venom in 1 c.c. NaCl 0.85 per cent. $9^h\ 55^m$ signs of paralysis, unable to walk. $10^h\ 10^m$ drowsy. $10^h\ 15^m$ unable to hold up head; breathing rapid. $10^h\ 20^m$ appears to be dying; reflexes absent. $10^h\ 25^m$ breathing labored, slower. $10^h\ 27^m$ dying; heart-beat slow and weak. $10^h 35^m$ occasional gasps; heart-beat not felt. $10^h 40^m$ stopped breathing, heart still beating, very weak.

Rabbit 4, 1000 g.

May 30, $1^h\ 34^m$ injected 0.1 c.c. venom into ear-vein. $1^h\ 35^m$ pupils contracted. $1^h\ 36^m$ lying down; heart-beat weak; breathing rapid. $1^h\ 37^m$ heart-beat weak, fast; reflexes present, pupils contracted. $1^h\ 38^m$ heart-beat weak, slow; breathing rapid, strained. $1^h\ 39^m$ pupils excessively contracted; ears bloodless. $1^h\ 40^m$ convulsions; pupils dilate, reflexes present. $1^h\ 45^m$ occasional respiratory gasps. $1^h\ 46^m$ reflexes still present; convulsions. $1^h\ 49^m$ reflexes absent; heart action slow. $1^h\ 50^m$ dead; chest opened; heart still beating; blood not clotted in heart; clots quickly after escape into chest cavity.

Guinea-pig 41, 600 g.

May 31, $12^h\ 00^m$ injected subcutaneously 0.1 c.c. venom in 1 c.c. NaCl 0.85 per cent. $12^h\ 30^m$ lies down. $12^h\ 40^m$ breathing rapid. $1^h\ 30^m$ falls on side. $2^h\ 00^m$ breathing very strained, right legs paralyzed. $2^h\ 30^m$ breathing difficult, slower. $2^h\ 45^m$ lying on side; tetanic convulsions when stimulated. $2^h\ 50^m$ dying. $3^h\ 10^m$ dead.

Rat 14, 98 g.

MAY 31, *12h 10m* injected subcutaneously 0.1 c.c. venom in 1 c.c. NaCl 0.85 per cent. *12h 30m* lying on side; breathing rapid. *12h 40m* lying on side; breathing slow. *12h 45m* reflexes present. *1h 00m* reflexes absent. *1h 10m* better; able to move about. *2h 00m* lies on side; apparently dying; reflexes present. *2h 50m* dead.

Dog 1, 10 kg.

MAY 31, *3h 14m* given subcutaneously 0.1 c.c. fresh venom. *3h 25m* vomited; urine and feces voided. *3h 30m* lying down; very sick. *3h 50m* vomited. JUNE 1, died during forenoon.

Dog 2, 9700 g.

MAY 31, *3h 17m* given subcutaneously 0.02 c.c. fresh venom. *3h 30m* lying down; trembles; occasionally much twitching. *3h 40m* voids urine and feces. Recovered.

Dog 3, 11 kg.

MAY 31, *3h 30m* given 1.1. c.c. fresh venom subcutaneously. *3h 45m* vomited; urine and feces voided. Very sick all afternoon. Died during night.

Cat 1, 3000 g.

JUNE 1, *12h 10m* give subcutaneously 0.4 c.c. fresh venom. *12h 20m* vomits. *12h 25m* pupils enormously dilated; vomits. *12h 30m* very nervous; cries if touched; breathing difficult. *2h 00m* breathing rapid, superficial, pupils still dilated. Sick for some days, but recovered.

Cat 2, 3500 g.

JUNE 1, *12h 08m* given subcutaneously 0.09 c.c. of fresh venom. *12h 25m* pupils enormously dilated. *12h 40m* breathing difficult. *2h 00m* breathing rapid, superficial, pupils dilated. Recovered.

Cat 3, 3000 g.

JUNE 1, *12h 06m* given subcutaneously 0.045 c.c. of fresh venom. *12h 25m* pupils enormously dilated, vomits. *12h 40m* pupils contracting. Recovered. JUNE 24 sick, killed; no lesions.

Cat 4, 3000 g.

JUNE 3, *2h 12m* given 0.9 c.c. of fresh venom subcutaneously. *2h 20m* violent movements of head from side to side, cries, pupils enormously dilated, eyes roll. *2h 35m* more quiet; eyes still roll, but head movements stopped. *2h 55m* lies quiet, very nervous; makes crying movements, but has nearly lost voice; breathing difficult, audible. *3h 06m* no longer able to hold up head; voice gone. JUNE 4 living; takes no food. JUNE 6 dead.

Cat 7, 1500 g.

JUNE 8, *12h 55m* injected intraperitoneally 0.6 c.c. of fresh venom. *1h 05m* pupils greatly dilated, losing voice. *1h 40m* pupils contracted, voice gone; breathing labored; tears secreted. Recovered.

Cat 9, 700 g.

JUNE 8, *2h 25m* injected intraperitoneally 0.7 c.c. of fresh venom. *2h 35m* very much affected; pupils dilated; tears and saliva flowing; breathing forced. *5h 00m* has remained in apparently dying condition all afternoon; breathing forced; nearly paralyzed. *5h 30m* dead.

CONDITION OF MOUSE'S EYE PRODUCED BY INJECTION OF HELODERMA VENOM.

We found that the injection of heloderma venom into mice caused frequently a peculiar condition of the eye. This condition did not appear as a rule when mice were injected with a large quantity of venom—a quantity which was lethal within a comparatively short time—but appeared usually when quantities of venom had been injected which caused death after many hours or several days. At times the injection of a sublethal dose of venom was sufficient to produce this lesion. However, its appearance was not constant, even after the injection of a lethal dose.

The lesion was observed as early as from 1½ to 6 hours after the injection of the venom. At this time the eyeball appeared somewhat more prominent than usual and the cornea showed a slight opacity. The protrusion of the eye and opacity of the cornea progressed fairly rapidly (there was, however, con-

siderable variation in the rapidity of advance of these symptoms), until the eye protruded about 2 or 3 mm. from its socket and the cornea assumed a gray-white color. This appearance was observed as early as 3 hours after the injection of venom.

If the condition continued to progress the gray-white of the cornea and sclera now turned red and gradually assumed a dark red color. At times the blood which had collected in the tissue about the eye-ball would break through the conjunctiva and a more or less free oozing of blood about the eye would be the result. Whether this breaking through of the blood was due to the pressure exerted by the blood within the eye-socket, or whether it was due to external trauma, we can not state. It is certain that when the cornea has become absolutely opaque the animal is blind, and, in view of the blindness and the unusual prominence of the eye, the mouse might easily rub the weakened eye against some object and thus injure the conjunctiva in moving about the cage.

In those cases in which the condition progressed only as far as the stage of corneal opacity, the animal seemed to be able to regain its vision after several days; but when the condition progressed to the stage of subconjunctival hemorrhage or actual oozing of blood, the eye-ball was lost, although the animal might otherwise recover from the injections. The stages of healing, either the return to normal or the disappearance of the protrusion and hemorrhage, progressed usually very rapidly, so that while the lesion usually reached its maximum severity between 12 and 24 hours after the injection, no external evidence of the lesion could be noted at a period 3 days after the injection.

It remained to be determined whether these macroscopic appearances represented a pathological condition within the eye-ball or within the periocular tissue. We removed the eye-ball and periocular tissue of several mice which had been injected with venom and which showed the various stages of the conditions described above. These eyes were sectioned and studied microscopically.

The microscopic examination of these tissues showed that in the earliest stages of the condition there was a slight extravasation of blood in the loose fatty tissue around the optic nerve, usually at some distance from where it entered the eye-ball, but no evidences of any intraocular lesions were observed. In later stages the hemorrhage in the periocular tissue became more evident and spread forward in close proximity to the optic nerve and also laterally in the fat tissue. Gradually the blood worked around the eye-bulb and appeared under the conjunctiva. At no time was any blood found inside the eye-bulb. The only abnormal condition within the eye consisted in the forward pressing of the lens until it almost touched the posterior surface of the cornea. It is possible that this condition of the lens was due to the manipulation of the eye during its removal, or, perhaps, to the osmotic movements of the aqueous humor during the fixation of the tissue, since it was noted in only a few of the eyes examined.

These ocular lesions in mice that follow the injection of venom are, therefore, confined to the periocular tissue of the orbital space. There occurs first

a hemorrhage in the posterior portion of the orbital space which gradually increases. The eye-ball is thus pressed forward and exophthalmus develops. In some unexplained manner the cornea becomes opaque. The blood works its way forward in the periocular tissue until it appears under the conjunctiva and this may at times, as a result of either the internal pressure or the trauma of the conjunctiva, produce conjunctival oozing.

The cause for the primary hemorrhage in the posterior portion of the orbital space is not clear. We have no evidence which would point to any vascular lesions causing a greater permeability of the vessels for the red blood-cells. The probable explanation must, therefore, be sought in an increase of the intravascular pressure within the vessels of the brain and orbit sufficiently great to cause a rupture of the thin and rather poorly supported vessels of the orbit.

A somewhat similar condition has been noted by Emmert* in mice which were injected with adrenalin. He found that when adrenalin was injected subcutaneously into mice, the eye-ball protruded and the cornea became opaque, but this author makes no mention of any hemorrhage. He also observed that the lens was pressed forward.

ANATOMICAL LESIONS PRODUCED BY INJECTION OF VENOM.

The most conspicuous and common of the macroscopic changes to be seen after death are found in the alimentary canal. These are particularly marked if some hours have elapsed between the injection of venom and the animal's death. The serous layer of the intestines and stomach is usually markedly congested. The intestines are much dilated with fluid and gas. In guinea-pigs and rabbits—the only mammals investigated with reference to this point—the mucous layer of the stomach was congested and showed small hemorrhages and areas of self-digestion. To determine beyond question that these changes were produced by the venom we made the following experiments: The abdominal cavity of four guinea-pigs was opened and the condition of the intestines noted. The abdominal wall was then sewed up and three animals were injected subcutaneously with varying doses of venom and one with the same volume of physiological salt solution. Immediately after death the intestines of the animals injected with venom were found to contain much more fluid and gas than they had contained before injection and also to show the other symptoms previously mentioned. The animal which had been injected with salt solution was killed and found to have undergone no evident alteration of the alimentary canal. Two control guinea-pigs belonging to the same lot were killed and likewise showed no abnormalities of stomach or intestine.

Inasmuch as certain of the snake venoms are perhaps excreted through the gastric mucous membrane, it might be thought that the post-mortem changes observed in this organ were due directly to a digestive action of the heloderma venom. In order to see whether similar changes might not be produced by other methods of slow poisoning, we killed guinea-pigs by injecting sublethal doses of potassium cyanide and of resorcin, repeating the injections

*Emmert, Virchow's Archiv, 1908, 194, 114.

at intervals, so that the animals continued seriously affected until death finally resulted. By means of both these poisons hemorrhages and self-digestion of the stomach were produced. The self-digestion was not considerable in the case of potassium-cyanide poisoning, but it was marked when death was caused by resorcin. Particularly in one case, where the poisoning extended over more than 24 hours, the mucous membrane was so changed that it came away entire from the muscular layers.*

After death following the injection of venom the lungs always showed more or less congestion and edema. These were the only invariable naked-eye changes that we observed. At times liver, spleen, kidneys, and adrenals showed congestion, but not very markedly and not invariably. Contrary to the experience of Santesson, we never saw the slightest evidence of any local disturbances. At the point of injection there was neither hemorrhage nor swelling, or any other symptom of local effect.

Santesson records these local symptoms in frogs, and in order to observe whether they might be specific in these animals we injected frogs with doses of both fresh and dried venom, and of strengths sufficient to kill in long, short, and medium intervals of time, but in no case did we observe any lesions at the place of injection, either before or after death. We were also unable to confirm Santesson's experience that the muscles at the site of injection become softened and easily disintegrated, although we looked again and again for evidence to this effect.

In all the reports concerning the local effect of the bite of the *Heloderma* in man, mention is made of the rapid appearance of swelling and hemorrhagic discoloration of the skin at the site of injury. We found that when fresh venom was injected subcutaneously into an animal, no swelling or hemorrhage appeared at the site of injection; and when venom was injected intramuscularly, no hemorrhage or swelling was noted. These negative results were obtained with venom collected in the usual manner, namely, by allowing the animal to bite on a piece of soft rubber and collecting only the venom which appeared between the rubber and the lower lip of the animal. In some other experiments we collected venom by allowing the animals to chew small pieces of sponge; the venom was squeezed and washed out of these sponges. The venom thus obtained was injected subcutaneously into the ear of a rabbit and into the tissue-pad of a rat's paw. Both of these animals developed marked swellings at the site of inoculation, and later showed ecchymoses and hemorrhages in these areas.

It is possible that the local symptoms—swelling and hemorrhage—result from some buccal secretion of the *Heloderma*. They certainly are not caused by the secretion of the true venom glands. It is impossible, however, to absolutely rule out mechanical injury as a factor in causing the appearance of these local symptoms when an individual is bitten by a *Heloderma*. The animal has very powerful jaws and its bite would easily bruise a large area of flesh and skin. When the animal bites it clings tenaciously, and in endeavoring to extricate a wounded part the injury might easily be increased.

Cf. the paper by M. E. Rehfuss, which presents a more elaborate experimental study of these lesions.

The possibility that the local symptoms are due to the effects of microorganisms which enter the wound either at the time of biting or after the biting can not be excluded, since in our experiments the venom obtained by allowing the helodermas to chew the sponges was not sterilized before injections.

The following records of autopsies performed on animals which had been injected with heloderma venom show some of the appearances which have been mentioned above:

Rabbit 13.

JUNE 26, $2^h\ 05^m$ received in peritoneal cavity 0.2 c.c. of venom. $5^h\ 10^m$ dead. Autopsy immediately; lungs, hemorrhagic and edematous; spleen, congestion and hemorrhages; hemorrhagic points in omentum; blood in heart fluid; hemorrhagic points in stomach; much fluid in small intestine.

Rabbit 14, 2 kg.

JUNE 26, $3^h\ 05^m$ received subcutaneously 0.4 c.c. of venom. $5^h\ 40^m$ dead. Autopsy immediately; lungs congested, hemorrhagic and edematous; ventricles not contracted; small intestine contains much fluid; stomach shows self-digestion.

Rabbit 16, 2 kg.

JUNE 26, $3^h\ 29^m$ received subdurally 0.2 c.c. of venom. $5^h\ 30^m$ dying; killed. Autopsy immediately; lungs edematous and emphysematous; spleen hemorrhages and congestion; ventricle not contracted; much fluid in small intestine; stomach shows hemorrhages and traces of self-digestion.

Rabbit 17, 1890 g.

JUNE 26, $3^h\ 57^m$ received intravenously 0.2 c.c. of venom. $4^h\ 45^m$ dead. $5^h\ 10^m$ autopsy; auricles still beating; lungs hemorrhagic and edematous; ventricle not contracted; small hemorrhages of the stomach.

Guinea-pig 105, 240 g.

JUNE 26, $2^h\ 40^m$ received intraperitoneally 0.1 c.c. of venom. $3^h\ 20^m$ dead. Autopsy immediately; fluid in intestine slightly increased; hemorrhages in stomach.

Guinea-pig 107, 220 g.

JUNE 26, $2^h\ 20^m$ received intraperitoneally 0.05 c.c. of venom. $3^h\ 00^m$ dead. Autopsy immediately; fluid in intestine slightly increased; hemorrhages in stomach.

Guinea-pig 109, 220 g.

JUNE 26, $2^h\ 45^m$ received intraperitoneally 0.02 c.c. of venom. $5^h\ 10^m$ dead. Autopsy immediately; much fluid in intestine; hemorrhages and self-digested areas in stomach.

Guinea-pig 106, 240 g.

June 26, $2^h\ 42^m$ received subcutaneously 0.1 c.c. of venom. $3^h\ 20^m$ dead. Autopsy; fluid in intestine slightly increased; hemorrhages in stomach.

Guinea-pig 108, 220 g.

JUNE 26, $2^h\ 22^m$ received subcutaneously 0.5 c.c. of venom. $3^h\ 25^m$ dead. Autopsy immediately; fluid in intestine considerably increased; hemorrhages in stomach.

Guinea-pig 110, 220 g.

JUNE 26, $2^h\ 48^m$ received subcutaneously 0.2 c.c. of venom. $6^h\ 05^m$ killed; appeared likely to die before morning. Autopsy immediately; fluid abundant in intestine; hemorrhages and self-digested areas in stomach.

Guinea-pig 111, 260 g.

JUNE 26, $4^h\ 10^m$ received subcutaneously 0.1 c.c. of venom. $5^h\ 20^m$ dying; killed. Autopsy; fluid slightly increased in intestine.

Guinea-pig 112, 260 g.

JUNE 26, $4^h\ 15^m$ received intraperitoneally 0.1 c.c. of venom. $5^h\ 10^m$ dead. Autopsy immediately; fluid slightly increased in intestine; small hemorrhages in stomach.

Guinea-pigs 114 and 115 (controls).

JUNE 26, $6^h\ 15^m$ from same lot; killed for controls; showed no abnormal fluid content of intestine, no hemorrhages, and no self-digested areas of stomach.

Guinea-pig 99, 400 g.

JUNE 21, $2^h\ 00^m$ opened abdominal cavity and examined intestines; sewed up wall and injected subcutaneously 0.05 c.c. of venom; no symptoms. $2^h\ 35^m$ injected 0.1 c.c. of venom. $5^h\ 00^m$ dead. Autopsy; intestines contain much more fluid and gas than at previous examination; hemorrhage in omentum; serous layer of intestine slightly congested; mucous membrane of stomach hemorrhagic; small self-digested areas.

Guinea-pig 100, 420 g.

JUNE 21, 2^h 18^m opened abdominal cavity, examined intestines and sewed up wall; injected 0.1 c.c. of venom subcutaneously. 5^h 15^m dead; fluid and gas increased in intestine; hemorrhages and self-digested areas in stomach.

Guinea-pig 102, 420 g.

JUNE 21, 2^h 50^m opened abdominal cavity, examined intestine, sewed up wall, and injected 0.15 c.c. of venom. 5^h 30^m dead; fluid and gas increased in intestine; hemorrhages and self-digested areas in stomach lining.

Guinea-pig 101, 440 g. (control).

JUNE 21, 2^h 40^m opened abdominal cavity, examined intestines, sewed up wall, and injected subcutaneously 1 c.c. of sterile salt solution. 5^h 40^m killed; no changes.

Guinea-pigs 103 and 104 (controls).

JUNE 21, 6^h 10^m killed two guinea-pigs from same lot for controls; intestines without excess of fluid or gas; stomachs normal.

HISTOLOGICAL CHANGES PRODUCED BY INJECTION OF VENOM.*

We investigated the histological changes produced in the organs of rabbits, guinea-pigs, and mice by the injection of heloderma venom. In some cases the animals had died shortly after a single injection, in other cases the organs examined were from animals which had received repeated injections of venom over varying periods of time. In the animals which died of acute poisoning, after a single administration of venom, no distinct histological changes were noted in any of the organs. Some of these animals died but a few minutes after the injection (rabbits injected intravenously with large doses), while others lived as long as 48 hours after the injection (rabbits or guinea-pigs in whose abdominal cavity a collodion capsule containing venom had been placed).

In the organs of animals dying a very short time after the administration of venom—within 30 minutes—no changes whatsoever were noted. When the animals died at periods between 1 hour and 48 hours after the administration of the venom the only noticeable change was a general venous congestion in the liver, lungs, and kidneys. In no cases were any degenerative changes noted in the organs of rabbits, guinea-pigs, or mice.

In the animals which received repeated injections† of venom over long periods of time, changes were occasionally found in the various organs, but these changes did not appear regularly, nor did they bear any relationship to the length of time during which the animal had been injected with venom or to the amount of venom injected.

The livers of none of the animals showed any degenerative changes. In one liver, from a rabbit which had received injections for a period of more than 6 months and which during this time had received 758 mg. of venom (150 mg. at the last injection), a slight increase of the connective tissue about the portal vessels was noted and in places this connective tissue was pushing in between the liver-cells. The liver of another rabbit which had received in all 0.96 c.c. of venom during a period of almost 3 months showed a slight increase of the connective tissue about the portal vessels. The livers of the other animals injected showed no changes.

The kidney of one rabbit which died after having received 1,050 mg. of venom during a period of almost 6 months showed an increase in the number of

*The microscopical examination of the various organs has been carried out by Dr. M. S. Fleisher.

†These were animals which we had attempted to immunize against heloderma venom by the administration of gradually increasing doses of venom. The results of these immunization experiments are reported later.

connective-tissue cells between the tubules and about the glomeruli. The epithelium of the tubules was swollen and in many places obliterated their lumen. The nuclei of some of these cells showed karyolysis and in some tubules nuclei were seen lying free in the lumen. The glomeruli of this kidney showed no distinct signs of involvement. One other kidney removed from an animal which had received 67.5 mg. of venom during a period of 3 months showed a slight increase of connective-tissue cells between the tubules. The kidneys of the other animals examined appeared normal.

All the hearts examined were normal, except one which showed a slight increase of connective tissue between the muscle-fibers and also a marked degree of vacuolization in the muscle-fibers over a rather small area. Elsewhere the muscle-fibers of this heart were normal. This animal, in which also a connective-tissue overgrowth in the kidney was found, had received 67.5 mg. of venom during a period of 3 months.

The lungs of several of the rabbits showed pneumonic areas. In these areas the capillaries were congested and the alveoli contained desquamated epithelium, polymorphonuclear leucocytes, and considerable granular detritus. In the lungs showing this condition the large vessels were dilated with blood. It is probable that this pneumonic condition represents simply a terminal infection and can not be attributed directly to the influence of the venom.

The bone-marrow and spleen of animals that had received a number of injections of venom showed more constant changes than did the other organs. In bone-marrow, which showed no gross change, it was generally noted that the giant cells were increased in number (in four or five cases); the polymorphonuclear leucocytes seemed to be increased in number, while the erythroblasts showed a relative decrease. The histological picture suggests at least that there was an effort to regenerate leucocytes or to produce a leucocytosis, and this latter explanation is in agreement with the blood-picture noted in animals which received repeated injections of heloderma venom.

The spleens of the rabbits which had received several injections of venom showed several types of changes, one or more of which appeared in the spleen of every animal examined. Most commonly we noted a change in the malpighian corpuscles. These appeared smaller than normal and the cells forming these bodies were less densely packed about the small vessels; in short, the malpighian corpuscles were less prominent than normal. Almost as regular as this change in the malpighian corpuscles was the proliferation of the endothelial cells in the splenic pulp. This proliferation of the endothelial elements of the splenic pulp was at no place very marked, but appeared diffusely throughout the spleen. In a few cases we noted the splenic pulp to be less dense and cellular, and as a result of this thinning of the splenic pulp the connective-tissue framework—especially the finer trabeculæ—became more prominent. The cellular elements of the spleen pulp did not show any marked changes; no one type of cell appeared to be unduly increased or diminished.

Thus we see that in the liver, kidney, and heart the venom does not produce any typical histological changes, but, when injected repeatedly over a

long period of time, it may lead to a slight degree of fibrosis in these organs. In the lungs the venom itself does not produce any distinct changes and, as stated above, the pneumonic condition which has been noted is most probably due to a terminal infection. In the bone-marrow, however, changes are produced which probably represent an effort to produce leucocytosis of the polymorphonuclear element.

INFLUENCE OF THE METHOD OF ADMINISTRATION ON THE TOXICITY OF THE VENOM.

It was possible in certain cases to determine exactly the influence that a certain mode of administration of the heloderma venom might have on the toxic effect, and especially upon the lethal dose of the venom. We have tested the influence of injecting the venom subcutaneously, intravenously, intraperitoneally, and subdurally, and also of injecting it into the stomach or intestines.

We found that the venom acts most quickly when injected intravenously, and also that, when thus administered, the minimal lethal dose is smaller than when administered in any other manner. When injected in this manner the venom causes the death of the animal in a very short time, usually within 30 minutes.

The effect of the venom appears rather more slowly when it is injected intraperitoneally, and the amount necessary to cause the death of the animal is distinctly larger than when injected intravenously.

Although the subcutaneous injection of venom kills the animals rather more slowly than does the intraperitoneal injection, it is probable that very little difference exists between the smallest amount of venom necessary to kill the animal when injected in these two different methods. As a rule, the minimal lethal dose appears to be the same, but occasionally an animal injected subcutaneously would survive the injection, whereas an animal injected intraperitoneally with a similar quantity of venom died. The most important difference between the effects of injecting venom subcutaneously and intraperitoneally probably arises from the fact that the absorption of venom from the peritoneal cavity is more rapid than the absorption from the subcutaneous tissues.

When injecting venom subdurally, one difficulty to be met and overcome is the probability that the injection of large quantities of fluid into the subdural spaces may, of itself, have some injurious influence. Although we injected 1 c.c. of 0.85 per cent sodium-chloride solution subdurally in guinea-pigs without producing any noticeable effects, it is possible that the injection of 1 c.c. of the diluted venom solution may produce more harmful effect than the injection of 0.1 c.c. of pure venom solution. Indeed, our experiments would appear to support this possibility. When guinea-pigs were injected subdurally with venom which had been diluted (they received subdural injections of 1 c.c. of fluid), they died in almost as short a time as the animals which were injected intravenously, and the minimal lethal dose appeared to be approximately the same whether administered intravenously or subdurally. On the other hand,

when very small quantities (between 2 and 8 drops) of the fresh undiluted venom were administered subdurally to rabbits we found that animals injected subcutaneously with similar quantities of venom died as soon or sooner than those which were injected subdurally. Whether these contradictory results represent a difference between the reactions of guinea-pigs and rabbits to the heloderma venom, we can not state, but it appears most probable that the differences are to be explained by the injection of the large quantity of fluid in the experiments with the guinea-pigs. It therefore seems that when venom is injected subdurally it is no more active than when injected subcutaneously. Perhaps the dilution of the venom and the quantity of fluid injected determine the rapidity with which the ganglia-cells are affected, and correspondingly the time of death.

When venom was injected directly into the stomach of a guinea-pig, by opening the abdominal wall and passing the needle of the syringe through the wall of the stomach, no effects due to the injection were observed. The same negative result was noted when venom was injected into the small intestines. Whether no venom is absorbed from the stomach and intestines, or whether the venom is so changed by the gastric or intestinal secretions that it has lost its toxic properties, we must leave undecided.* Similarly, venom of Colubridæ has very little or no effect when introduced into the intestinal tract, while venom of Viperidæ causes intense irritation of the intestinal mucosa.

Yet another method of administrating the venom was tested by placing collodion sacs containing venom in the peritoneal cavity of rabbits and guinea-pigs. The results of these experiments will, however, be reported in another place.

We used 124 animals in testing the influence of the method of administering the venom, and the following examples have been selected as typical cases:

Subcutaneous injection: Guinea-pig 58, 400 g. JUNE 12, $3^h\ 55^m$ injected 0.1 c.c. of venom. JUNE 13, died during night.

Intraperitoneal injection: Guinea-pig 57, 560 g. JUNE 12, $3^h\ 56^m$ injected 0.1 c.c. of venom. Died during night.

Intravenous injection: Guinea-pig 64, 440 g. JUNE 12, $5^h\ 05^m$ injected 0.1 c.c. of venom. $5^h\ 30^m$ dead.

Subcutaneous injection: Guinea-pig 60, 420 g. JUNE 12, $3^h\ 50^m$ injected 0.05 c.c. of venom. No symptoms.

Intraperitoneal injection: Guinea-pig 59, 680 g. JUNE 12, $3^h\ 54^m$ injected 0.05 c.c. of venom. No symptoms.

Intravenous injection: Guinea-pig 65, 680 g. JUNE 12, $4^h\ 55^m$ injected 0.05 c.c. of venom. $5^h\ 25^m$ dead.

Subcutaneous injection: Guinea-pig 62, 640 g. JUNE 12, $3^h\ 39^m$ injected 0.02 c.c. of venom. No symptoms.

Intraperitoneal injection: Guinea-pig 61, 660 g. JUNE 12, $3^h\ 47^m$ injected 0.02 c.c. of venom. No symptoms.

Intravenous injection: Guinea-pig 66, 680 g. JUNE 12, $4^h\ 40^m$ injected 0.02 c.c. of venom. $5^h\ 20^m$ dead.

Subcutaneous injection: Guinea-pig 51, 740 g. JUNE 12, $9^h\ 30^m$ injected 0.2 c.c. of venom. $1^h\ 40^m$ dead.

Intraperitoneal injection: Guinea-pig 52, 620 g. JUNE 12, $9^h\ 33^m$ injected 0.2 c.c. of venom. $1^h\ 40^m$ only moderately affected. JUNE 13, recovered.

*Compare the chapter on the biochemistry of the venom, by Dr. Alsberg. It is not probable that the injurious influence of peptic and tryptic digestion upon the venom is sufficient to account for the lack of any symptoms after this mode of application of venom. Other factors, as adsorption by the contents of stomach or intestines, imperfect penetration through the wall of the alimentary tract, probably cooperate.

Intravenous injection: Guinea-pig 63,600 g. JUNE 12, $9^h\ 52^m$ injected 0.05 c.c. of venom. $10^h\ 05^m$ dying. $10^h\ 09^m$ dead.

Intravenous injection: Guinea-pig 54, 600 g. JUNE 12, $10^h\ 16^m$ injected 0.02 c.c. of venom. Showed serious symptoms, but recovered.

Subcutaneous injection: Rat 71, 170 g. JUNE 11, $11^h\ 45^m$ injected 0.2 c.c. of venom. Showed serious symptoms for a while and continued drowsy during day. JUNE 12, recovered.

Intraperitoneal injection: Rat 75, 190 g. JUNE 11, $11^h\ 46^m$ injected 0.2 c.c. of venom. Serious symptoms during day. Worse than rat 71. JUNE 12, dead.

Intravenous injection: Rat 79, 30 g. JUNE 11, $3^h\ 01^m$ injected 0.2 c.c. of venom. $3^h\ 15^m$ dead.

Subcutaneous injection: Rat 72, 170 g. JUNE 11, $11^h\ 48^m$ injected 0.1 c.c. of venom. Symptoms same as Rat 71. JUNE 12, recovered.

Intraperitoneal injection: Rat 76, 190 g. JUNE 11, $11^h\ 20^m$ injected 0.1 c.c. of venom. Serious symptoms. JUNE 12, recovered.

Intravenous injection: Rat 80, 120 g. JUNE 11, $3^h\ 20^m$ injected 0.1 c.c. of venom. $4^h\ 20^m$ dead.

Subcutaneous injection: Rat 73, 170 g. JUNE 11, $11^h\ 52^m$ injected 0.05 c.c. of venom. No symptoms. JUNE 12, living.

Intraperitoneal injection: Rat 77, 170 g. JUNE 11, $11^h\ 55^m$ injected 0.05 c.c. of venom. No symptoms. JUNE 12, living.

Intravenous injection: Rat 81, 130 g. JUNE 11, $5^h\ 15^m$ injected 0.05 c.c. of venom. $6^h\ 00^m$ dead.

Subcutaneous injection: Rat 74, 170 g. JUNE 11, $11^h\ 58^m$ injected 0.02 c.c. of venom. No symptoms. JUNE 12, living.

Intraperitoneal injection: Rat 78, 170 g. JUNE 11, $12^h\ 00^m$ injected 0.02 c.c. of venom. JUNE 12, living; no symptoms. $10^h\ 30^m$ second injection of 0.28 c.c. of venom. $1^h\ 30^m$ dead.

Intravenous injection: Rat 82, 125 g. JUNE 11, $5^h\ 20^m$ injected 0.02 c.c. of venom. JUNE 12, living.

Subcutaneous injection: Guinea-pig 92, 400 g. JUNE 21, $11^h\ 00^m$ injected 0.15 c.c. of venom. $1^h\ 15^m$ dead.

Intraperitoneal injection: Guinea-pig 95, 300 g. JUNE 21, $12^h\ 30^m$ injected 0.15 c.c. of venom. $1^h\ 15^m$ dead.

Subcutaneous injection: Rabbit A 1, 1500 g. FEB. 3, $10^h\ 30^m$ injected 15 mg. venom. FEB. 4, seems well. FEB. 6, dead.

Subdural injection: Rabbit A 2, 1500 g. FEB. 3, $2^h\ 30^m$ injected 8 mg. venom. FEB. 4, seems well. FEB. 7, dead.

Stomach injection: Guinea-pig 117, 340 g. JUNE 28, $3^h\ 15^m$ injected about 1 c.c. of venom in 6 c.c. NaCl. $4^h\ 10^m$ recovered from influence of ether; appears normal. JULY 1, living, normal.

Subcutaneous injection: Rabbit A 5, 2500 g. FEB. 3, $3^h\ 00^m$ injected 8 gtt. fresh venom. $3^h\ 30^m$ affected. $5^h\ 00^m$ improved.

Subdural injection: Rabbit A 6, 2100 g. FEB. 5, $4^h\ 30^m$ injected 8 gtt. fresh venom. FEB. 6, dead.

Stomach injection: Rat 112, 140 g. JUNE 28, $3^h\ 35^m$ injected 1 c.c. undiluted venom. Tied up stomach at point of puncture. $4^h\ 10^m$ appears well. JULY 1, living, normal.

Subcutaneous injection: Rabbit A 9, 1080 g. FEB. 9, $12^h\ 30^m$ injected 4 gtt. fresh venom. $3^h\ 00^m$ affected. $9^h\ 00^m$ dead.

Subdural injection: Rabbit A 10, 1100 g. FEB. 9, $12^h\ 45^m$ injected 4 gtt. fresh venom. $1^h\ 30^m$ slightly affected. FEB 10, $2^h\ 00^m$ dying; killed.

Stomach injection: Rat 111, 120 g. JUNE 28, $12^h\ 39^m$ injected 1 c.c. pure venom. $12^h\ 45^m$ respiration disturbed. $1^h\ 00^m$ improved, but remains lying down. $2^h\ 00^m$ somewhat better. $4^h\ 00^m$ somewhat better; may live. JUNE 27, dead.

Subcutaneous injection: Rabbit A 14, 1120 g. FEB. 13, $12^h\ 00^m$ injected 2 gtt. fresh venom. $2^h\ 00^m$ affected. $2^h\ 30^m$ dead.

Subdural injection: Rabbit A 13, 1120 g. FEB. 12, $12^h\ 00^m$ injected 2 gtt. fresh venom. $2^h\ 00^m$ much affected. $4^h\ 45^m$ dead. The symptoms produced in this experiment we attribute to the escape of venom from the wound into the peritoneal cavity. With rat 117, where precautions were taken to ligature the stomach at the point of injection, no symptoms appeared.

Subdural injection: Guinea-pig 93, 400 g. JUNE 21, $11^h\ 20^m$ injected 0.15 c.c. of venom. $11^h\ 28^m$ dead.

Subdural injection: Guinea-pig 94, 350 g. JUNE 21, $12^h\ 05^m$ injected 0.1 c.c. of venom. $12^h\ 13^m$ dead.

Subdural injection: Guinea-pig 96, 350 g. JUNE 21, $12^h\ 05^m$ injected 0.1 c.c. of venom. $2^h\ 45^m$ dies, having been almost dead since $2^h\ 07^m$.

Subdural injection: Guinea-pig 98, 350 g. JUNE 21, 2h 29m injected 0.05 c.c. of venom. 2h 45m dead; almost dead since 2h 32m.

Subdural injection: Guinea-pig 97, 350 g. JUNE 21, 2h 30m injected 1 c.c. normal saline. 4h 45m normal; killed.

Injection into intestines: Rabbit B 3, 1700 g. JUNE 28, open abdominal cavity; inject into intestines (small) 40 mg. venom. JUNE 29, well and lively. JULY 7, well and lively.

Injection into intestines: Rabbit B 4, 1600 g. JUNE 28, open abdominal cavity; inject into intestine (small) 20 mg. venom. JUNE 29, well and lively. JULY 7, well and lively.

EFFECT OF PLACING COLLODION CAPSULES CONTAINING VENOM IN THE PERITONEAL CAVITY.

In several rabbits and guinea-pigs we tested the influence of venom placed in the peritoneal cavity in collodion capsules. By this method we wished to determine to what extent venom passed through the collodion capsule in the animal body, and also the effect of venom being added continuously in very small quantities.

Eight rabbits and two guinea-pigs were used in these experiments. The collodion sacs were made by pouring a thin coating of collodion into a small test-tube and removing this collodion coating after the ether and alcohol had evaporated. The sacs were first tested with water and only capsules that were perfect were used. The solution of either fresh or dry venom was placed in the capsule, the neck of which was tied. The tied end of the capsule was further sealed with collodion and the capsule containing the venom was placed for an hour in a water-bath, the temperature of which was maintained at 80° C. Thereupon the capsule was put into the peritoneal cavity under the usual aseptic precautions.

We found the venom gradually passed out of the capsules and was absorbed by the animals. The lethal effect was proportionate to the quantity of venom put within the capsule. The number of capsules placed within the peritoneal cavity appeared to influence the toxic effect. The greater the number of capsules, the greater was the aggregate surface of diffusion through which the venom could pass out of the capsules.

Only two guinea-pigs were used in these experiments. One collodion capsule was placed in the peritoneal cavity of each of these animals; in one case the capsule contained 0.5 c.c. of fresh venom, in the other 24 mg. of dissolved dry venom. The first animal died after 28 hours, while the second died in 24 hours.

Both animals showed marked weakness about 12 hours after the capsules had been placed in the peritoneal cavity. The respiration was rapid and usually shallow. They were very quiet and did not move about their cages unless disturbed. From this stage they both passed on to a stage of distinct paralysis, in which the respiration was deep and strained. Previous to this latter stage, however, the guinea-pig which had received the capsule containing 24 mg. of venom developed marked convulsions which lasted for a short time and were immediately followed by the paralytic stage. The animals did not show any recovery from this dyspneic, paralytic condition; they died from 30 to 60 minutes after the appearance of this condition.

The post-mortem appearances were essentially the same in both animals. There was a slight amount of gelatinous exudate on the visceral peritoneum where the collodion sacs had been in contact with the intestines. The peritoneum elsewhere was smooth and not injected. The intestines contained considerable quantities of fluid and the stomach showed several small hemorrhagic and ulcerated areas.

The collodion sacs in both cases contained at the time of the autopsy considerably less fluid than had been put into them at the beginning of the experiment. It was, however, definitely shown that some venom still remained inside the capsules. They were washed out with sodium-chloride solution and various quantities of the solution were injected into mice. It is impossible to state exactly how much venom was found in the sacs, but the quantity was sufficient to kill some of the injected mice. In the case in which 24 mg. of venom had been put into the capsule, approximately 3 mg. were found at the end of the experiment; while in the other case only a very small quantity, possibly 0.02 c.c., of venom was recovered (about one-twentieth of the amount originally introduced).

Of 8 rabbits, 2 received one capsule (1 of these later received three capsules), 2 received two capsules, 3 received three capsules, and 1 received four capsules.

The first of the animals, receiving in one capsule 30 mg. of venom, died 9 days after the operation. The other rabbit received in one capsule 50 mg. of venom, but did not die; 25 days after the first capsule had been placed in its peritoneal cavity, it received three more capsules, each containing 0.3 c.c. of fresh venom. The animal died 2 hours after these last capsules had been placed in the peritoneal cavity.

The three animals with two capsules received each 60 mg. of venom, 30 mg. being in each capsule. One of these animals died 5 days afterwards, another in 2 weeks, while the third survived.

In the experiments in which three capsules were placed in the peritoneal cavity each capsule contained 0.3 c.c. of fresh venom, each rabbit receiving 0.9 c.c. of venom. One of these animals died in 40 hours, the others in 18 hours.

One rabbit, given four capsules each containing 0.3 c.c. of venom, died in about 12 hours.

All of the animals which survived the administration of venom for several days or weeks showed a marked loss of weight, which amounted in some cases to as much as one-third of the original body-weight; otherwise, they showed no symptoms of the poisoning, as they were usually active and their appetites continued good. Death usually occurred suddenly. In a period of a few hours the animal would develop weakness and prostration and death would follow rapidly. Evidently a gradual accumulation of venom takes place in the animal organism. The animals which died shortly after the capsules had been placed in the peritoneal cavity showed signs of weakness soon after the operation. Prostration and dyspnea appeared rapidly and the animals appeared to die as a result of respiratory failure.

In none of the rabbits were any especial changes found at the autopsy. In one animal, however, which died 18 hours after receiving three capsules, each containing 0.2 c.c. of venom, small hemorrhages and a few ulcers were noted in the gastric mucosa. The capsules were always found to be surrounded by a thick fibrinous exudate, which appeared to be partly organized in the cases in which the animals survived.

In two cases we made hemoglobin, red blood-cell, and leucocyte counts before and several days after the giving of the capsules. The results of these are given below:

Rabbit.	No. of capsules.	Amount of venom.	Period.	Hemoglobin.	Red blood corpuscles.	Leucocytes.
		mg.		*p. ct.*		
A 4	1	30	Before.....	15.66	6,430,000	8,160
			6 days after.....	16	6,430,000	18,040
A 8.....	1	50	Before.....	10.90	5,420,000	9,120
			24 days after.....	8.78	3,030,000	9,200

These experiments are too few in number and the results are too irregular to allow of any definite conclusions. It appears, however, that this method of administering venom has not a very marked effect on the elements of the blood.

ADMINISTERING FRACTIONAL DOSES OF VENOM.

In pigeons and mice small sublethal doses of venom were repeatedly injected in order to determine the rapidity with which the venom was destroyed by or eliminated from the body. For this purpose we used six pigeons. The following table shows the amounts injected, the time which elapsed between the injections, and the ultimate result:

Influence of injection of fractional doses of venom into pigeons.

First injection.	Time elapsed.	Second injection.	Time between second injection and death.	Result.
mg.	*hrs.*	*mg.*	*hrs. min.*	
0.3	3	0.3	.. ..	Recovered.
.4	3	.4	.. ..	Do.
.5	3	.5	.. ..	Do.
.6	3	.6	2 00	Died.
.6	2	.6	1 00	Do.
.6	1	.6	40	Do.

From these experiments we learn that when the dose of venom usually lethal for pigeons (0.8 mg.) is injected in fractional parts, with an interval of 3 hours between the injections, the animal survives the injection. If slightly more than the minimal lethal dose be injected, with a similar interval of time between the two injections, the pigeons still may survive. If, however, within a period of 3 hours, as much as 12 mg. of venom be injected, thus half again as much as the minimal dose, the pigeons die. When the interval between the two injections of venom is shortened to an hour the animal dies more quickly than when a longer time elapses between the two injections. It therefore

appears that in 3 hours the pigeon is able to eliminate or destroy a part, but only a relatively small part, of the injected venom.

The results of similar experiments with mice were not as regular as those with the pigeons.

Influence of administration of venom in fractional doses to mice.

First injection.	Time elapsed.	Second injection.	Time elapsed.	Third injection.	Death after—
mg.	*hrs. min.*	*mg.*	*hrs.*	*mg.*	
0.04	1 00	0.04	..	....	3 hours.
.04	1 00	.04	..	....	8 hours.
.04	3 00	.04	..	....	12 hours.
.04	3 00	.04	..	....	12 hours.
.08	4 00	.04	..	....	Recovered.
.04	1 00	.04	2	0.04	Do.
.04	2 00	.04	1	.04	8 hours.
.04	2 00	.04	2	.04	Recovered.
.04	1 00	.04	2	.04	Do.
.03	.. 45	.05	..	....	Do.
.03	.. 45	.05	..	....	Do.
.03	.. 45	.05	..	....	Do.

It has been noted before that the reaction of the individual mouse to the heloderma venom varies quite markedly and that frequently mice will die as a result of the injection of a dose which usually would prove to be sublethal. It seems that such has been the case in these experiments. While 0.15 to 0.12 mg. of venom may be considered as the lethal dose for mice, we find that some mice which received only 0.08 mg. of venom died as a result of the injection, even though 3 hours intervened between the injection of the two parts. On the other hand, some mice which in two or three injections received a quantity of venom equal to the minimal lethal dose survived. In view of these latter experiments, it appears probable that the injection of a minimal lethal dose in fractional parts does not necessarily cause the death of the mouse; we must, however, consider that 0.12 mg. is not necessarily a lethal dose.

In the case of the pigeon, within a few hours a relatively very small part of the venom is either eliminated or somehow transformed into such a condition that it is no longer injurious to the sensitive parts of the central nervous system of the injected animal.

These results agree with the effect we observed in animals in which the venom had been introduced by means of collodion capsules. Here also, owing to the slow diffusion of the venom, the lethal effect was much delayed and in some cases the animals, which would have died if the same amount had been injected directly, recovered.

SUSCEPTIBILITY OF VARIOUS SPECIES OF ANIMALS TO HELODERMA VENOM.

We have tested the susceptibility of warm-blooded vertebrates, cold-blooded vertebrates, and invertebrates to the venom of the *Heloderma*. In comparing the resistance of the warm-blooded vertebrates to heloderma venom we have used as a standard for comparison the minimal lethal dose per kilogram of body-weight.*

*We have not compared the differences in functional disturbances, as these seemed to bear but little relationship to the minimal lethal dose. In certain cases the animals were severely affected by the injection of venom, but were relatively resistant to its lethal effect.

We have, except in one case, compared only the minimal lethal doses of the dissolved dry venom. Although we have investigated the minimal lethal doses of the fresh venom, we have not, as a rule, used the results of these experiments in comparing the susceptibility of the various species, mainly on account of the already mentioned marked daily variation in the strength of the fresh venom. It has, however, been necessary in certain cases to compare also the lethal doses of the fresh venom in order to supplement the results as shown by the lethal doses of the dry venom, and in one case (cat) we have tested only the lethal dose of fresh venom. We have noted considerable variations in resistance among individuals of the same species, most especially when fresh venom was injected. Dogs showed a considerable individual variation in regard to sensitiveness, and in a lesser degree rabbits and mice showed similar variations. The variability among animals of the same species showed itself always in the direction of a resistance considerable below the average. We did not find individuals showing more than the average resistance.

We note that among the warm-blooded animals tested pigeons are the least resistant. The other animals all show relatively less susceptibility to the lethal effect of venom in approximately the following order: dog, guinea-pig, mouse, rabbit, cat, and rat. The dog, guinea-pig, mouse, and rabbit all seem to have approximately the same degree of resistance to the venom, since the lethal dose of the dry venom is very nearly the same for every kilogram of body-weight of each of these animals. The rat, however, shows distinctly greater resistance to the lethal effect of the venom than any of the other warm-blooded animals.

Minimal lethal doses of venom for warm-blooded animals.

Subject.	Average weight.	Absolute quantities.		Per kilogram of body-weight.	
		Fresh venom.	Dry venom.	Fresh venom.	Dry venom.
	gm.	*c.c.*	*mg.*	*c.c.*	*mg.*
(1) Pigeon.....	250	0.02	0.8	0.08	3.2
(2) Dog.....	10,000	0.1	100	0.01	10+
(3) Guinea-pig.....	500	0.05	5	0.1	10
(4) Mouse.....	15	0.005	0.15	0.25	10
(5) Rabbit.....	1,500	0.05	18	0.04	12
(6) Cat.....	3,000	0.9	.....	0.3	....
(7) Rat.....	125	0.1	5	0.8	40

It will be noted that the lethal dose of dried venom is approximately the same for every kilogram of body-weight of either dog, guinea-pig, mouse, or rabbit, the lethal dose being approximately 10 mg. for each of these animals. We do not, however, find a similarity regarding the lethal dose of fresh venom for these four classes of animals. The dog appears to be most susceptible to fresh venom; the rabbit, pigeon, guinea-pig, mouse, and rat follow in the order named. It is probable that this apparent variation in susceptibility to fresh venom is due largely to the variation in the toxicity of the venom.*

*In order to obtain the dry venom, many specimens of fresh venom were mixed; thus the dry venom represents a substance of approximately average toxicity. On account of the great variability in the toxicity of fresh venom not much importance can be attached to the minimal lethal quantities of fresh venom in the case of dogs, in which the number of experiments was necessarily small.

The lethal dose of the fresh venom only was tested in cats. We found that a cat weighing 3,000 gm. was killed by the injection of 0.9 c.c. of venom; thus the minimal lethal dose was 0.3 c.c. of venom pro kilogram of the body-weight. This dose is considerably larger than the lethal dose of fresh venom for dogs, guinea-pigs, or rabbits, and slightly larger than the lethal dose for mice.

Rats were, as stated above, distinctly more resistant to the injection of venom than any of the other animals. A rat weighing 125 gm. was killed by the injection of 5 mg. of dry venom or 0.1 c.c. of fresh venom. Thus the lethal dose pro kilogram of body-weight for rats was 0.8 c.c. of fresh venom and 40 mg. of dry venom.

Cold-blooded verbebrates are distinctly less susceptible to the action of venom than warm-blooded vertebrates. We have tested the lethal effect of venom of frogs, toads, snakes, turtles, eels, heloderma, and fundulus.

The *Heloderma* shows a resistance toward its own venom which for purposes of protection at least is complete. The injection of 2.25 c.c. of fresh venom—a quantity sufficient to kill 45 guinea-pigs each weighing 500 grams—produces no evident symptoms on the *Heloderma*, which weighs on the average 500 grams. A definite explanation accounting for this marked resistance of the *Heloderma* to the toxic effect of its venom can not be given at present, but in view of the results of experiments in which the adsorptive power of heloderma organs for heloderma venom was tested, it appears that the liver, and, to a less degree, the kidney, of the *Heloderma* exert a specific adsorptive action on the venom and this may explain, in part at least, the resistance of the *Heloderma* to its venom.* This resistance on the part of the *Heloderma* is not due to antibodies in the serum of the *Heloderma*, since we have found that venom which had been mixed with heloderma serum had not lost any of its toxic power. The relative immunity of poisonous animals against their own venom is evidently a general phenomenon.

We have found in a single experiment that the immunity of *Heloderma* toward its own poison does not extend to the snake venoms. The heloderma reacts very quickly toward rattlesnake venom, but, as we had for the determination of this point, only a small supply of snake venom of unknown strength, we can say no more concerning the lethal dose of this venom for helodermas than that it appears not to greatly exceed the lethal dose for rats and guinea-pigs.

The resistance of the snake to the toxic action of heloderma venom was tested in only two animals, both water-snakes (*Utania certalis*). One animal which weighed 80 grams died after the injection of 35 mg. of venom, while the other survived the injection of 25 mg. and only died after the second injection of a similar quantity. The lethal dose of venom for snakes appears to be approximately 400 mg. pro kilogram of body-weight.

The toad (*Bufo americanus*) shows a marked resistance to the toxic effect of venom. An animal weighing 50 grams was killed by the injection of 15 mg. of dry venom; thus for toads the lethal dose of venom pro kilogram of body-weight would be 300 mg., a quantity far greater than the lethal dose of venom

Cf. the chapter on the adsorption of venom, by M. Fleisher and Leo Loeb.

pro kilogram for rats, which proved to be the most resistant of the warm-blooded vertebrates tested. The toads showed very profound respiratory disturbances and almost complete paralysis for some hours following the injection of venom, but in spite of these alarming symptoms, unless the amount injected was 15 mg. or more, the animals recovered from the injection.

As is well known, the toad is resistant to a number of poisons besides the one contained in its own skin secretion. Certain of these poisons, notably antiarin and the poisons of the digitalis group, produce physiological disturbances similar to those produced by toad venom, and it has been suggested that the same mechanism by which the toad is able to protect itself against the latter poison answers also as a protection against the former ones. However that may be, the venom of *Heloderma*, toward which the toad shows also remarkable resistance, has physiological effects quite different from those produced by antiarin, digitalis, and the toad poisons; consequently, the same explanation will not suffice here. We made no experiments to investigate the nature of this resistance, as our supply of toads was limited.

Frogs (*Rana climatans* and *Rana pipiens*) proved to be more susceptible to the action of venom than toads. Five frogs weighing between 80 and 85 grams died after injection of 4, 5, and 6 mg. of venom; one frog weighing 84 grams received 5 mg. of venom and survived; two frogs weighing 80 and 75 grams, respectively, survived after injection of 3 mg. of venom; 4 mg. represents, therefore, approximately the lethal dose for a frog weighing 80 grams.

Tadpoles were found to be more susceptible than adult frogs. Tadpoles weighing approximately 2 grams survived after the injection of 0.025 mg. and died after injection of 0.05 mg. of venom. The lethal dose for a kilo of tadpoles is, therefore, between 12 and 25 mg. of venom, while the lethal dose pro kilogram of frogs is 50 mg.

Lethal doses of venom for cold-blooded vertebrates.

Animal.	Average weight in grams.	Lethal dose, in milligrams.	Lethal dose pro kilo., in milligrams.	Largest dose injected which was not lethal, in cubic centimeters.
Snake	80	35	425	....
Toad	50	15	300	....
Frog	80	4	50	....
Turtle	80	5	65	....
Heloderma	500	...	...	2.25
Eel	(?)	...	...	.1
Fundulus	(?)	0.1	...	....

Testing the susceptibility of various kinds of turtles to the heloderma venom, we found that all reacted in relatively the same manner to the venom. We used the spotted terrapin and painted terrapin in most experiments, but also used the stink-turtle and mud-turtle. The injection of 5 mg. of venom was found sufficient to cause the death of a turtle weighing 80 grams. The lethal dose of venom for a turtle is, therefore, 65 mg. for every kilogram of body-weight.

We tested the effect of venom on only one eel; this animal, which weighed 100 grams, was not affected by the injection of 0.1 c.c. of fresh venom. Whether this is nearly the minimal lethal dose we can not state.

The fundulus (*F. majalis*) was the only member of the fish family in which the influence of venom was tested. We found that 0.1 c.c. of fresh venom was the minimal lethal dose for these animals. Since the fundulus is a small fish weighing probably 30 grams, it is evident that the lethal dose pro kilogram would be very large, possibly 3 c.c. of venom.

Our investigations on invertebrate animals indicate that these animals are immune to heloderma venom, or, if not immune, they at least possess a resistance enormously greater than vertebrate animals. We injected individuals of *Sycotopus canaliculatus*, *Limulus*, *Nereis virens*, *Phascolosoma gouldii*, *Asterias forbesii*, *Mnemiopsis leidyi*, and *Gonionemus murbachii*, without being able to produce evident symptoms in any of these animals, although we injected them with doses sufficient to kill more than 50 times the same weight of guinea-pig. We also demonstrated in the case of *Gonionemus* that animals immersed in sea-water containing 5 per cent of sterile venom continued their swimming movements unchanged for many hours and died only after a length of time sufficient for bacterial decomposition to render the sea-water unsuitable. Controls placed in sea-water plus 5 per cent of sterile human saliva died somewhat later, but as the sea-water plus venom contained far more decomposable material than the sea-water plus saliva, no conclusion concerning the toxicity of venom for *Gonionemus* could be drawn from this circumstance. We likewise found that fertilized sea-urchin and starfish eggs placed in sea-water containing 3 to 7 per cent of venom continued to develop and formed a number of swimming gastrulæ. That the number which reached this stage in the venom solution was considerably less than in controls is not surprising, when we consider the large amount of organic matter and the consequent putrefactive changes taking place in the sea-water containing venom.

Maximum quantities of venom injected into invertebrates.*

Animal.	Maximum quantity.	
	c.c.	
Limulus	0.6	No toxic effects.
Sycotopus	0.4	
Phascolosoma	0.3	
Asterias	0.3	
Mnemiopsis	0.3	
Nereis	0.25	
Gonionemus	0.05	

TOXICITY OF THE ORGANS AND EXCRETIONS OF HELODERMA.

Suspensions of the various organs of the *Heloderma* were injected in order to determine whether any organ other than the venom gland secreted or held the venom. The various organs tested were the liver, kidney, spleen, pancreas, and lachrymal gland (?). (This latter was a gland which lay on the lower portion of the eye-socket.) The bile, urine, and blood-serum were also tested.

*Where in our report the dose of venom is given in volume and not in weight, fresh venom was used; otherwise, dried venom.

In making the suspensions, the various organs were first minced and crushed and then added to a very small quantity of 0.85 per cent sodium-chloride solution. This mixture was allowed to stand for about 2 hours before being injected into the animals. In all cases the effects of the organ extracts were tested on mice.

We found that the injection of 1 c.c. of the suspension of kidney, pancreas, and spleen of *Heloderma* had no visible effect upon the animals. Two mice injected with liver suspension, one with 1 c.c. the other with 0.5 c.c., died on the third day after the injection, in all probability not as a result of the injection, but due to starvation. Two other animals injected with 1 c.c. and 2 c.c. of liver suspension, respectively (not at the same time as the other two), showed no ill effects. Three animals were injected with heloderma egg which had been mixed with an equal quantity of sodium-chloride solution; they received either 0.5 c.c., 1 c.c., or 2 c.c. of this mixture and none of them was affected by the injection. Several animals which received variable doses up to 1 c.c. of the suspension of the supposed lachrymal gland or in which pieces of this organ were placed in a subcutaneous pocket remained perfectly well. From the above experiments it would appear that none of the organs of the *Heloderma*, including the gland which is found in the eye-socket, have a toxic action.

Two mice were injected with urine of the *Heloderma;* one received 1 c.c., the other 2 c.c. Neither of these animals showed any disturbances following the injection.

Two mice were injected with serum of the *Heloderma.* Both received 2 c.c. of serum, but no toxic effect was evident.

One mouse injected with 0.25 c.c. of heloderma bile died after 2 hours, while another mouse which received 0.1 c.c. of bile survived the injection. In order to test the toxicity of bile, we injected a mouse with 0.5 c.c. of dog's bile and this animal died in 2 hours. It is evident that the toxic effect of the heloderma's bile is due not to any content of venom, but to some substance contained in bile, such as the bile-salts. In order to further show that the toxic effect of the heloderma's bile was not due to venom, we injected some mice with various quantities of bile and venom and other mice with like quantities of venom alone. The mice which received the injections of bile and venom did not die sooner than those which received the venom alone. On the other hand, the addition of bile to the injected venom did not diminish the toxicity of the venom, since the mice which received like quantities of venom died approximately the same length of time after the injection, irrespective of whether bile had or had not been added.

These results obtained with the organs and also with the eggs of *Heloderma* differ markedly from those obtained with the blood of snakes and certain other animals possessing venom-secreting glands. The blood of the latter has by various investigators* been found to be very poisonous; while the blood of *Heloderma* is innocuous. It is possible that the toxicity of certain organs of the

*Phisalix et Bertrand, Archiv. de Physiol. 1894; Calmette, Compt. Rend. de la Soc. Biol., 1894, x, ser. I; Wehrmann, Annales de l'Institut. Pasteur, XI, 810, 1897.

Crotalus, observed by Flexner and Noguchi,* was entirely or partially due to the admixture of blood in the organs. These authors, as well as Phisalix,† established the toxicity of the ova of venomous snakes, while we found the ova of *Heloderma* to be devoid of any toxic effect. We may add that Fraser‡ described an antivenomous action of the bile of serpents.

INFLUENCE OF VENOM ON COAGULATION TIME OF THE BLOOD.

The influence of the *Heloderma* venom on the coagulation time of the rabbit's blood was studied in various ways. The first method consisted in injecting large quantities of venom intravenously and shortly before the death of the animal, collecting the blood through a glass canula into a small test-tube. We injected intravenously doses of venom of 20 to 30 mg., an amount causing the death of the animal in periods varying from 5 to 10 minutes. The coagulation time for the blood in these cases varied from 4 to 11.5 minutes, apparently independent of the amount of venom injected or the interval between the injections and the taking of the blood. In one case, a large quantity of blood collected in a flask coagulated in 12 minutes.

Since all the rabbits injected with venom died as a result of asphyxia (death being due to respiratory failure), we considered it necessary to determine what influence asphyxia itself might have on the coagulation time of the blood. We therefore compressed the trachea of a rabbit which was deeply under the influence of ether and after 10.5 minutes of intermittent compression bled the animal. The blood collected in a small test-tube coagulated in 4 minutes; that collected in a small flask in 12 minutes.

The results of these experiments lead to the conclusion that the venom of the *Heloderma* has no distinct influence on the coagulation time of the blood.

Influence of injection of venom on the coagulation of the rabbit's blood.

Rabbit No.	Weight.	Amount of venom injected.	Interval between injection and taking of blood.	Coagulation time.
	gms.		*h. m.*	*h. m.*
613	1100	30 mg.....	10 00	4 00
616	1440	25 mg.....	7 30	11 00
617	1280	20 mg.....	4 45	5 45
618	1700	15+5+5 mg.....	5 00	4 30
				[a]12 00 (in flask)
619	1200	Asphyxia.....	10 30	4 00 12 00 (in flask)

[a]After last injection.

The second method of testing the influence of venom on the coagulation time of the blood was to allow blood to flow through a canula into a tube containing various quantities of venom solution. In these experiments we used 0.5 c.c., 1 c.c., 2 c.c., and 3 c.c. of a 1 per cent solution of the dry venom to 2 c.c. of blood. In every case the fluid in the test-tubes was made up to 5 c.c. with 0.85 per cent sodium-chloride solution. Furthermore, two control experi-

*Jour. Path. and Bacteriol., VIII, 1903. †Compt. Rend. Soc. Biol., 1905, LVII.
‡British Med. Journ., I, 1897.

ments were made with the blood of the same animal, in which 2 c.c. of blood were added to 3 c.c. of sodium-chloride solution and one in which 5 c.c. of undiluted blood were collected. The coagulation times of the various mixtures are given below:

Influence of venom on the coagulation of the rabbit's blood.

Blood.	1 per cent venom solution.	NaCl solution.	Coagulation time.
c.c.	c.c.	c.c.	h. m.
2	0.5	2.5	15 00
2	1.0	2.0	6 15
2	2.0	1.0	4 00
2	3.0	...	13 30
2	...	3.0	19 40
2	...	3.0	13 15
5	...	...	5 40

From these experiments it is again evident that venom neither delays nor accelerates noticeably the coagulation of the blood.

Inasmuch as in the preceding experiment the individual variations were marked and the quantities of venom used were large, another similar experiment was performed. Into a series of test-tubes each containing 2 c.c. of venom dissolved in 0.85 per cent NaCl solution 2 c.c. of rabbits' blood was allowed to flow from a canula inserted into the carotid artery.

Injection.	Beginning coagulation.	Surface of blood coagulated.	Complete coagulation.
	h. m.	h. m.	h. m.
2 c.c. rabbit's blood plus 2 c.c. 0.85 per cent NaCl	6 20	.. ..	10 40
2 c.c. rabbit's blood plus 2 c.c. 0.85 per cent NaCl containing 1 mg. venom	4 05	6 40	10 35
Do	4 25	8 15	9 50
2 c.c. rabbit's blood plus 2 c.c. 0.85 per cent NaCl containing 2 mg. venom	4 00	7 05	9 50
Do	3 50	.. ..	8 40
2 c.c. rabbit's blood plus 2 c.c. 0.85 per cent NaCl containing 7 mg. venom	4 05	7 20	8 50
Do	5 35	.. ..	7 50
2 c.c. rabbit's blood plus 2 c.c. 0.85 per cent NaCl containing 12 mg. venom	3 00	.. ..	10 00
2 c.c. rabbit's blood plus 2 c.c. 0.85 per cent NaCl	6 40	.. ..	11 55

The individual variations in this experiment, due to imperfections of technique, are relatively slight. There is little doubt that the admixture of venom caused an insignificant acceleration of the coagulation that is not specific. Addition of a chemically inert foreign body in fine suspension would have had at least an equally strong accelerating effect.

We also used a third method, which had been employed by Noc in his studies of the influence of snake venoms on the coagulation of the blood. For this we obtained 100 c.c. of rabbit plasma kept liquid through the addition of 1 gram of sodium citrate. This citrate plasma coagulates readily after addition of $CaCl_2$.

So much 0.85 per cent NaCl solution was added that the total volume in each tube was 2 c.c. It was found that 0.4 to 0.6 c.c. of 5 per cent $CaCl_2$ caused a coagulation of this plasma in approximately 14 to 15 minutes. After

addition of 1, 2, 3, 4, 5, and 7 mg. of venom dissolved in 1 c.c. of 0.85 per cent NaCl to 1 c.c. of citrate plasma, but without $CaCl_2$ the plasma remained liquid for 24 hours, when the experiment was interrupted.

Plasma.	0.5 per cent $CaCl_2$ added.	Beginning of coagulation.	Complete coagulation.
c.c.	*c.c.*	*h.* *m.*	*h.* *m.*
1	0.2	20 20	30 00
1	0.3	15 40	19 50
1	0.4	9 00	14 30
1	0.5	13 00	15 8
1	0.6	10 50	13 20
1	0.7	14 10	16 10

If we add venom to the citrate plasma and afterwards 0.4 c.c. 0. 5 per cent $CaCl_2$ the various quantities of venom again do not exert any specific influence on coagulation.

1 per cent citrate plasma.	0.5 per cent $CaCl_2$ added.	Venom ndded.	Beginning of coagulation.	Complete coagulation.
c.c.	*c.c.*	*mg.*	*h.* *m.*	*h.* *m.*
1	0.4	1	8 15	13 45
1	0.4	2	8 5	14 5
1	0.4	3	7 55	13 55
1	0.4	5	6 55	14 50
1	0.4	7	9 45	14 35
1	0.4	10	10 55	14 35
1	0.4	..	6 55	11 35
.	...	..	7 00	.. ..

In each case the venom had been dissolved in 0.85 per cent NaCl solution and to each test-tube, so much 0.85 per cent NaCl was added that the total volume of fluid in the test-tube was 3 c.c. In this case addition of venom caused a quite insignificant delay in the coagulation of the citrate plasma which, however, did not bear any direct relation to the amount of venom added. From all these experiments we may conclude that venom does not exert any distinct influence on the coagulation of the blood.

Our results differ from those obtained by van Denburgh and Wight, who state that they found the venom of *Heloderma* exerting a distinct influence on the coagulation of the blood. In order to clear up, if possible, the cause of this divergence in the results obtained by ourselves and by van Denburgh and Wight, who in some of their experiments had used fresh venom, while we worked with solutions of dried venom, we made a few additional experiments in which we injected into the ear-veins of rabbits an extract of the venom gland of *Heloderma* after it had been filtered through filter paper. The extract was prepared by rubbing up 0.87 gram of gland with 6 c.c. of 0.85 per cent NaCl solution.

Fifty seconds after the injection of 0.5 c.c. of the extract into a rabbit weighing 800 grams, the animal was in a dying conditon; 30 seconds later blood was obtained by making an incision into the heart. Respiration had ceased at that time, but the heart was still beating. Some of the dark-colored blood was kept in a porcelain dish; the rest was distributed into four test-tubes,

each receiving 1 to 2 c.c. blood. After 1 minute 40 seconds there was the beginning of coagulation in the tubes and 8 minutes after the withdrawal of the blood the coagulation in the test-tubes was completed; 3 minutes later the blood in the porcelain dish was a solid coagulum. In a control experiment we withdrew in a similar manner, directly after death, the blood from a rabbit whose neck had been broken. In this case the blood escaped more slowly from the heart and consequently came into a prolonged contact with the tissues. Coagulation here was completed in 1 minute 25 seconds.

In a third experiment the same venom-gland extract was used after it had been standing on ice for several days; 30 seconds after the intravenous injection of 1 c.c. of the extract, the rabbit, weighing about 2,200 grams, was weak and its respiration forced; 3 minutes after the first injection a second injection of 1 c.c. of venom was given; 6 minutes after the second injection the animal died and the blood was withdrawn through an incision into the heart; after 1 minute 10 seconds the blood was coagulated in the porcelain dish and almost coagulated in the test-tubes. In the latter the blood had been shaken during the process of coagulation and the clot somewhat retracted. In consequence of this premature retraction some blood remained fluid in the test-tubes; this blood became solid 3 minutes after the formation of the first clot.

We may conclude from these experiments that after injection of the gland extract an exceedingly slight retardation in the coagulation of the blood may take place. It is, however, very doubtful whether this retardation is to be attributed to a specific effect of the venom; it is more probably due to the influence of tissue extract, which produces a negative phase if injected in very small quantity. However that may be, at the best the effect of the injection of gland extract on the coagulation of the blood was very small in our experiments.

In contradistinction to the venom of *Heloderma* various snake venoms have a pronounced action on the coagulation of the blood, some venoms accelerating, others inhibiting it.

INFLUENCE OF INOCULATION OF PIECES OF VENOM GLAND INTO MICE.

In the course of some work concerning the growth of pieces of the venom gland transplanted into helodermas, the retention of the toxicity of the venom in the transplanted pieces of gland was also studied. In order to determine this, pieces of venom gland were inoculated into mice. The details of the transplantation of these pieces of gland will be found in a previous paper dealing with the morphology. It will be sufficient here to state that pieces of the gland were placed under the skin of the same animal's thorax and at the same time under the skin of another animal's thorax, so that each animal had two grafts. The first mentioned piece will be spoken of as the auto-transplant, the second as the iso-transplant. Four experiments were carried out in which the pieces were removed 1, 2, 3, and 4 weeks respectively after the transplantation, and were then inoculated into mice. The pieces of venom gland were placed under the skin of the mice by means of a trocar and canula in a manner similar to that by which the routine mouse-tumor inoculations are made.

Before giving the results of the experiments with the transplanted glands we will give the results of some experiments in which fresh venom glands were inoculated into mice, as well as some in which the juice expressed from the fresh gland was injected.

Two mice were inoculated with small pieces of heloderma venom gland which had just been removed. Both of these mice died about 2 hours after being inoculated.

A small quantity of the fluid expressed from the cut surface of a fresh venom gland was measured and diluted with 0.85 per cent sodium-chloride solution and various quantities of this mixture were injected into mice. The results of the injection are given in the following table:

Injection of venom-gland fluid into mice.

Amount of venom-gland fluid injected.	Result.	Interval between injection and death.
c.c.		*h. m.*
0.01	Died	15
0.005	Died	1 00
0.002	Died	40
0.0003	Died	2 15
0.00006	Died	2 15
0.00001	Recovered	.....

The fluid from the venom gland was in this case about 100 times as strong as the venom obtained from the heloderma's mouth (since the lethal dose of the latter is 0.005 c.c. for a mouse). In another experiment 0.44 gram of a fresh gland was cut in small pieces and in a mortar mixed with 3 c.c. of 0.85 per cent NaCl. As much fluid as possible was pressed out of the gland and the mixture was filtered through a paper filter. 100 c.c. of 0.85 per cent NaCl solution were added to 1 c.c. of the filtrate. One mouse received 1 c.c. and another 0.5 c.c. of this diluted extract subcutaneously. Both mice were soon affected and died within less than an hour. Another mouse which received 0.1 c.c. of this diluted filtrate was found dead 2 days after the injection; mice that received still smaller doses remained alive. A mouse which received, therefore, the filtered juice of about 0.7 mg. of the venom gland died in less than an hour.

The experiments in which the toxicity of the pieces of venom gland transplanted subcutaneously into helodermas were tested gave the following results:

Inoculation of transplanted pieces of venom gland into mice.

Period after transplantation.	Effect of auto-transplant on mouse.	Effect of iso-transplant on mouse.
1 week	Died in 20 hours	Died in 5 hours.
2 weeks	Died in 44 hours	Died in 2 hours.
3 weeks	Died in 50 minutes	Died in 9 hours.
4 weeks	Died in 30 minutes	Died in 9 to 18 hours.

From these experiments it is evident that the fluid in the transplanted pieces of gland retains its toxic effect even after a period of 4 weeks. Whether there has been any diminution in the strength of this fluid we are unable to state.

In another experiment the piece of venom gland was placed under the skin into the subcutaneous tissue of a turtle's leg. Two days after the transplantation, when the turtle seemed to be dying, the piece of gland was removed; part was reserved for histological study and another part divided and inoculated into two mice. Both of these mice died 45 minutes after the inoculation. (The turtle died on the following day, that is, about 2.5 days after the gland had been placed under the skin.) Even in the turtle the fluid of the venom gland of the *Heloderma* retains its toxicity for a period of 2 days.

IMMUNIZATION AGAINST HELODERMA VENOM.

By giving repeated and constantly increasing doses of venom an attempt was made to immunize animals against the venom of the *Heloderma*. For this purpose rabbits, pigeons, and geese were used, the latter animals because they were reputed to be resistant to various venoms, and especially to certain bacterial infections.

In immunizing the geese, we injected into the pectoral muscles of the animals very small doses (0.05 c.c.) of the fresh venom. The first or second injection appeared to produce no effect, so that the quantity of venom was increased to 0.07 c.c., and in every case the animals died after the third or fourth injection. The attempt to immunize geese against venom was given up after all four geese used in these experiments had died.*

Immunization of rabbits against heloderma venom.

Rabbit.	Date of first injection.	Amount injected.	Date of death.	Amount last injection.	Total amount injected.	No. of injections.
X 1	Dec. 5, 1907	15 mg.	Apr. 20, 1908	150 mg.	1050 mg.	16
X 2	Dec. 5, 1907	15 mg.	Apr. 4, 1908	21 mg.	152 mg.	9
X 3	Dec. 10, 1907	7.5 mg.	Jan. 8, 1908	7.5 mg.	33 mg.	5
X 4	Jan. 7, 1908	7.5 mg.	Mar. 7, 1908	15 mg.	67.5 mg.	7
X 5	Dec. 21, 1907	7.5 mg.	Jan. 25, 1908	7.5 mg.	27.5 mg.	4
X 6	Jan. 20, 1908	7.5 mg.	Jan. 31, 1908	12 mg.	34 mg.	3
Y 1	June 18, 1908	.02 c.c.	June 23, 1908	.02 c.c.	.02 c.c.	1
Y 3	June 24, 1908	.02 c.c.	Aug. 10, 1908	.12 c.c.	62 c.c.	9
Y 4	Aug. 14, 1908	.05 c.c.	Oct. 5, 1908	.1 c.c.	.54 c.c.	7
Y 2	June 18, 1908	.02 c.c.	Oct. 16, 1908	.12 c.c.	.80 c.c.	11
Y 5	Aug. 20, 1908	.05 c.c.	Nov. 6, 1908	.14 c.c.	.96 c.c.	10
Z 1	Dec. 29, 1908	5 mg.	Apr. 1, 1909	17 mg.	135 mg.	13
Z 2	Do	5 mg.	Mar. 3, 1909	14 mg.	88 mg.	10
Z 3	Do	5 mg.	Feb. 11, 1909	10 mg.	51 mg.	7
Z 4	Do	5 mg.	June 15, 1909	150 mg.	758 mg.	23
Z 5	Do	5 mg.	May 14, 1909	38 mg.	233 mg.	17

With the ten pigeons used in these experiments we were somewhat more successful. 0.5 mg. of venom was given at the first injection, this dose producing no distinct effect. At periods varying from 1 to 33 days after this first injection a second injection of a like quantity of venom was given. Four pigeons died after the second injection of 0.5 mg. of venom. The six remaining pigeons received, 5 to 7 days after this injection, 0.6 mg. of venom, and thereafter, every 5 or 10 days, slightly increased doses of venom. Four of the remaining pigeons died; one after three, one after six, one after seven, and one after eight injec-

*In repeating these experiments, it will be advisable to use dried instead of fresh venom, in order to eliminate the variations in the strength of the venom as a complicating factor.

tions. All of these pigeons, excepting the one dying after the third injection, had received several injections of 1 mg. of venom, and it was following the injection of this amount of venom that they died. The two remaining pigeons received eight injections; the last injection being 1.1 mg. of venom. In view of the poor results obtained with the majority of pigeons we discontinued the experiments. It appeared that pigeons might survive one injection of a lethal dose of venom and die from the effect of a second or third injection of a like quantity.

In the experiments with rabbits we used in all 16 rabbits. The table on page 88 gives the total amounts injected, the period of time over which the injections extended, and the various doses of venom used.

It will be seen from the above protocols that the experiments in immunizing rabbits were successful in so far as the establishment of an active immunity against the venom is concerned. We were, however, unable to push this immunity to as high a degree as it appeared desirable. The rabbits would at times survive the injection of a large dose of venom and eventually die from the injection of an only slightly increased dose. Both the fresh venom and the dry venom were tried; but because of the variation in strength of the fresh venom, the dry venom was used in most cases.

The injections were given every 5 or 7 days, provided the animal did not show emaciation; if it had lost weight, no injection was given until the original weight had been regained. As a further precaution in the first few injections (in the experiments with rabbits Z1, Z2, Z4, and Z5), the venom was mixed with a 1 per cent solution of calcium hypochlorite, a method of starting the injections for purposes of immunization recommended by Calmette in his work with cobra venom. At first equal quantities of venom solution and calcium-hypochlorite solution were used, but gradually the quantity of hypochlorite solution was diminished and the amount of venom increased. The quantity of venom was increased very slowly until the amount injected was greater than the lethal dose; then the quantities injected were increased somewhat more rapidly.

Only four of the animals were injected with quantities of venom larger than the usual lethal dose (Rabbits X1, X2, Z4, Z5), and only two of these (X1 and Z4) received sufficient quantities to produce any distinct immunity; these two withstood the injection of about eight times the dose lethal for ordinary rabbits.

It is impossible to explain the death of the animals after the injection of an only slightly increased dose of venom or after the injection of a minimal lethal dose of venom. That we are not dealing with a typical anaphylactic reaction is evident from the special experiments in which we tested the anaphylactic power of venom. It appears that the animals' resistance to venom varied from time to time and that the immunity produced was not a definite and stable one. It not rarely happened that abscesses developed at the place of injection, notwithstanding all the precautions taken to insure a sterile procedure. It may be that after all conditions similar to the Arthus phenomenon of local

anaphylaxis were present. Other investigators have made similar observations in the immunization against snake venoms.*

Blood taken from two of the rabbits (X1 and Z4) was tested for the presence of precipitins. Furthermore, the protective action of the serum obtained from these animals was tested against venom and mixtures of venom and of serum obtained from the immunized rabbits were injected into mice.

PROTECTIVE POWER OF THE SERUM OF IMMUNIZED RABBITS.

Mice were used in testing the protective power of the serum of immunized rabbits.

Some blood was drawn from rabbit Z4 after it had withstood the injection of 95 mg. of venom in one dose and after it had been under treatment for four and a half months. This blood was allowed to clot and the serum was drawn off after the blood had stood in the ice-box over night.

Mice were injected with venom, venom and immune serum, and venom and normal-rabbit serum. Injection of 0.02 c.c. of fresh venom killed a mouse in 35 minutes, 1 c.c. of normal-rabbit serum added to the venom killed a second mouse in 20 minutes, while a mouse injected with a similar quantity of venom which had stood for an hour mixed with 1 c.c. of immune-rabbit serum died after 3 hours.

The results were practically the same when we first injected the serum and then the venom as when they were injected together. Thus control mice injected with either 0.015 or 0.01 c.c. of fresh venom died somewhat sooner than mice which received similar quantities of venom but which had, in addition, received 1 c.c. of either normal or immune-rabbit serum; the animals which received the immune serum lived a little longer than those which received the normal-rabbit serum in addition to the venom. When we injected 2 c.c. of serum, followed, in 40 minutes, by the injection of 0.01 c.c. of fresh venom, we found that the animal which received immune-rabbit serum and venom lived longer than a mouse which received venom alone, or a mouse which received 2 c.c. of normal-rabbit serum followed by 0.01 c.c. of fresh venom solution. This mouse died as soon as the control animal.

The same rabbit whose serum was tested in the above experiments (Z4) was bled again a few days later before any further injections of venom had been made. At this time only a very small quantity of blood was drawn. In all the experiments with this serum the mice received 0.01 c.c. of fresh venom. Two quantities of serum were used, namely, 1 c.c. and 1.5 c.c. of either the immune-rabbit serum or the normal-rabbit serum. All the six mice used in these experiments, those injected with venom alone as well as those injected with venom plus serum, died in approximately the same length of time.

The other rabbit (X1) was bled 4 months after the injections were begun, when it had developed sufficient resistance to withstand the injection of a dose of 100 mg. of venom. In these experiments mice were injected with venom alone, with venom and normal-rabbit serum, and with venom and immune-

*Immunization with heloderma venom might perhaps be more easily accomplished if it were possible to separate venom and proteid material through heating, as can be done in the case of cobra venom. This would obviate the necessity of injecting accessory proteids that may add to the local anaphylactic condition.

rabbit serum. All the mice which received 0.2 mg. of dry venom died approximately at the same time after the injection, even when the injected venom stood for an hour after having been mixed with 0.5 c.c. of immune serum. Similar results were obtained when 1.4 mg. of venom were injected, even though 1 c.c. and 2 c.c. of immune serum had been added.

The injection of 0.4 or 0.6 mg. of venom plus 1 c.c. of immune serum was not lethal when the venom and serum of the immunized rabbit were allowed to stand until a precipitate had formed, whereas the injection of the same quantities of venom without serum caused death.

We conclude from these experiments that although repeated injections of venom into rabbits enable them to resist the toxic effect of large doses of venom, no distinct antitoxin had been produced. It may be noted that in some cases the previous injection of immune-rabbit serum or the mixing of the venom with immune-rabbit serum before the injection of venom into mice delayed slightly the lethal effect of the venom, so that we may perhaps conclude that a very small amount of antitoxin had been produced. Had it been possible to carry the immunization of the rabbits further, an antitoxin might have been produced. It is, however, quite apparent that relatively enormous quantities of venom—more than we could devote to this problem—would have been required in order to accomplish the production of an antitoxin, and it was doubtful whether, even in the case of perfect success, the results would have been of sufficient theoretical interest to justify such an expenditure of venom and of effort.

Antitoxic power of the serum of rabbits immunized against heloderma venom.

Serum from rabbit.	Amount of venom.	Amount of immune serum.	Amount of normal serum.	Mouse died after—
Z 4*	0.02 c.c.	1 c.c.		2 hours.
	.02 c.c.		1 c.c.	20 minutes.
	.02 c.c.			30 minutes.
Z 4*	.015 c.c.	1 c.c.		12 hours.
	.015 c.c.		1 c.c.	12 hours.
	.015 c.c.			30 minutes.
Z 4*	.01 c.c.	1 c.c.		72 hours.
	.01 c.c.		1 c.c.	2 hours 30 minutes.
	.01 c.c.			2 hours.
Z 4*	01 c.c.	2 c.c.		6 hours.
	.01 c.c.		2 c.c.	3 hours.
	.01 c.c.			4 hours.
Z 4†	.01 c.c.	1 c.c.		1 hour.
	.01 c.c.		1 c.c.	1 hour 15 minutes.
	.01 c.c.			1 hour 15 minutes.
Z 4†	.01 c.c.	1.5 c.c.		2 hours.
	.01 c.c.		1 c.c	2 hours.
	.01 c.c.			2 hours.
X 1	.2 mg.	0.5 c.c.		6 hours.
	.2 mg.		0.5 c.c.	6 hours.
	.2 mg.			6 hours.
X 1	.08 mg.	0.5 c.c.		Recovered.
	.08 mg.		0.5 c.c.	Do.
	.08 mg.			Do.
X 1	.04 mg.	0.5 c.c.		Do.
	.04 mg.		0.5 c.c.	Do.
	.04 mg.			Do.
X 1	1.4 mg.	2 c.c.		30 minutes.
X 1	1.4 mg.	1 c.c.		30 minutes.
	1.4 mg.		1 c.c.	30 minutes.
	1.4 mg.			30 minutes.
X 1	0.4 mg.	‡1 c.c.		Recovered.
	0.4 mg.			1 hour.
X 1	0.6 mg.	‡1 c.c.		Recovered.
	0.6 mg.			1 hour.

*Rabbit bled May 23, 1909. †Rabbit bled May 27, 1909. ‡Standing 4 hours showed precipitate.

PRECIPITINS IN THE SERUM OF RABBITS IMMUNIZED AGAINST HELODERMA VENOM.

We mixed the blood of the rabbits which had been immunized against venom with various quantities of fresh venom, dried venom, or dried heloderma blood, and noted the appearance of precipitates in these mixtures. We used in these experiments the serum of two of the immune rabbits, X1 and Z4, at a time when they were able to resist an injection of 100 mg. and 95 mg. of venom, respectively. In the following table the results of these experiments are given:

Precipitins in serum of rabbits immunized against heloderma venom.

With Fresh and Dried Venom.				
Amount of serum.	0.2 mg.	0.4 mg.	0.6 mg.	Remarks.
1 c.c. immune*	+	+	+	
1 c.c. normal	trace	trace	trace	
0.1 c.c. immune*	+	+	+	Precipitate appears sooner than with 1 c.c. immune serum.
0.1 c.c. normal	trace	trace	trace	
0.05 c.c. immune*	+	+	+	
0.05 c.c. normal	trace	trace	trace	Precipitate appears sooner than with 0.1 c.c. immune serum.
	2 mg.	.2 mg.	.01 mg.	
0.25 c.c. immune†	+	+	+	
0.25 c.c. normal	0	0	0	
	0.1 c.c.	0.025 c.c.	0.0025 c.c.	
0.25 c.c. immune†	+	+	+	Somewhat heavier precipitate than with dry venom.
0.25 c.c. normal	0	0	0	
	2 mg.	0.2 mg.	0.01 mg.	
0.025 c.c. immune†	0	trace	0	
0.025 c.c. normal	0	0	0	
	0.1 c.c.	0.025 c.c.	0.0025 c.c.	
0.025 c.c. immune†	+	+	trace	
0.025 c.c. normal	0	0	0	
	2 mg.	0.2 mg.	0.01 mg.	
0.005 c.c. immune†	0	0	0	
0.005 c.c. normal	0	0	0	
	0.1 c.c.	0.025 c.c.	0.0025 c.c.	
0.005 c.c. immune†	+	+	0	
0.005 c.c. normal	trace	0	0	

With Dissolved Heloderma Blood which had previously been Dried.				
Amount of serum.	2 mg.	0.2 mg.	0.01 mg.	Remarks.
0.25 c.c. immune†	+	+	0	
0.25 c.c. normal	0	0	0	
0.025 c.c. immune†	+	0	0	
0.025 c.c. normal	0	0	0	
0.005 c.c. immune†	0	0	0	
0.005 c.c. normal	0	0	0	

*Serum of rabbit X 1. †Serum of rabbit Z 4.

The serum of the immunized rabbits contains venom precipitins. However, the serum of normal rabbits may also contain precipitins for the venom, but not in such large quantities as the immune serum. The serum of rabbit X1 contained more precipitin than the serum of rabbit Z4.

In view of the fact that the smaller quantities of serum reacted more rapidly with the venom than the larger quantities of serum (experiments with serum of rabbit X1), we may conclude that there is an optimum proportion for mixtures of the venom and immune serum. However, the degree of pre-

cipitation in the different tubes eventually becomes the same in all cases. Whether the precipitins owe their origin to and react with the venom proper or proteid material admixed to the venom, can not be decided without a determination of the chemical character of the venom.**

The serum of the immunized rabbits contains also a precipitin which reacts with the blood of the *Heloderma*. This reaction was, however, quite weak and only a small amount of precipitate was found in serum-blood mixtures. Whether this reaction is due to the same precipitin which reacts with the venom or to a specific precipitin for the heloderma blood is doubtful. There is a strong possibility that it is a specific heloderma-blood precipitin, since, as we have stated earlier, the venom frequently contained small quantities of blood which were dried and again dissolved simultaneously with the venom.

DOES BLOOD SERUM OF HELODERMA CONTAIN A VENOM ANTITOXIN?

The antitoxic properties of the serum of both normal helodermas and the helodermas from which the venom gland had been removed were tested. Before injection, the serum was allowed to stand mixed with the venom for periods of from 30 minutes to an hour at room temperature. These mixtures were then injected into mice and at the same time control mice were injected with the same quantities of venom which had not been mixed with serum.

Experiment No.	Injection.			Result.
	Venom.	Quantity of serum.	Kind of serum.	
No. 1	0.2 mg			Died in 4 hours.
2	0.2 mg	1 c.c	Glandless heloderma*	Died in 12 hours.
3	0.1 mg			Died in 4 hours.
4	0.1 mg	1 c.c	Glandless heloderma*	Died in 24 hours.
5	0.07 mg			Died in 48 hours.
6	0.07 mg	1 c.c	Glandless heloderma*	Recovered.
7	0.005 c.c	1 c.c	Normal heloderma	Died in 3 hours.
8	0.005 c.c	1 c.c	Glandless heloderma†	Died in 3 hours.
9	0.005 c.c			Died in 3 hours.
10	0.01 c.c	1 c.c	Normal heloderma	Died in 2 hours.
11	0.01 c.c	1 c.c	Glandless heloderma†	Died in 2 hours.
12	0.01 c.c			Died in 2 hours.
13	0.006 c.c	1 c.c	Normal heloderma	Died in 2 hours.
14	0.006 c.c	1 c.c	Glandless heloderma†	Died in 2 hours.
15	0.006 c.c			Died in 2 hours.
16	0.01 c.c	1 c.c	Normal heloderma	Died in 2 hours.
17	0.01 c.c	1 c.c	Glandless heloderma†	Died in 2 hours.
18	0.01 c.c			Died in 2 hours.
19	0.005 c.c	1 c.c	Normal heloderma	Recovered.
20	0.005 c.c	1 c.c	Glandless heloderma‡	Recovered.
21	0.005 c.c			Recovered.
22	0.01 c.c	1 c.c	Normal heloderma	Died in 4 hours.
23	0.01 c.c	1 c.c	Glandless heloderma‡	Recovered.
24	0.01 c.c			Died in 2 hours.
25	4 mg	1 c.c	Normal heloderma	Died in 45 minutes.
26	4 mg	1 c.c	Normal heloderma	Recovered.
27	4 mg	1 c.c	Normal heloderma	Recovered.
28	4 mg	1 c.c	Glandless heloderma‡	Recovered.
29	0.005 c.c	1 c.c	Guinea-pig§	Recovered.
30	0.01 c.c	1 c.c	Guinea-pig§	Recovered.
31	0.07 mg	1 c.c	Sheep¶	Died in 6 hours.
32	0.07 mg	1 c.c	Sheep¶	Died in 18 hours.
33	0.07 mg	1 c.c	Ox	Recovered.

* Poison glands had been removed 66 days previously (serum 1).
† Poison glands had been removed 30 days previously (serum 2).
‡ Poison glands had been removed 7 days previously (serum 3).
§ Injected at same time as mice injected with serum 3.
¶ Injected at same time as mice injected with serum 1.

**The results obtained by Dr. Alsberg make it almost certain that the precipitins formed owe their origin not to the injection of the venom proper but to admixed proteid material.

We may conclude from these experiments that the addition of normal heloderma serum or of the serum of a *Heloderma* from which the venom gland had been removed does not neutralize the venom. In a few cases the animals which were injected with venom mixed with serum of a glandless heloderma lived somewhat longer than controls injected with a similar quantity of venom; and two animals recovered, while the controls died. In other cases, no effect of the heloderma serum was noticeable. Inasmuch as guinea-pig serum and ox serum had in a few cases a similar effect, we can not attribute to the serum of *Heloderma*, when mixed with heloderma venom, any specific antitoxic effect.

We tested the serum of glandless helodermas, having in mind the possibility that the venom gland might discharge some venom into the blood and that the venom present in the blood might obscure a possible antitoxic property of the serum.

We may, therefore, conclude that the resistance of the *Heloderma* to the injection of large doses of venom is not due to any substance contained in the circulating blood.

DOES THE INJECTION OF VENOM PRODUCE A STATE OF ANAPHYLAXIS?

We injected pigeons, mice, and rabbits with heloderma venom, and several days or weeks later injected these animals a second time in order to determine whether any phenomena of anaphylaxis could be found.

Twelve pigeons were injected with doses of venom varying from 0.2 to 0.6 mg.; at various times from 4 to 33 days after the first, a second injection was given. Three animals died after the second injection, but in all these cases death was due to the administration of an overdose of venom. The nine remaining animals showed no ill effects whatever from the second injection. The results of these experiments are given in the following table:

Experiments in anaphylaxis (pigeons).

First injection.	Time elapsed.	Second injection.	Result.	First injection.	Time elapsed.	Second injection.	Result.
mg.	*days.*	*mg.*		*mg.*	*days.*	*mg.*	
0.6	4	0.8	Died.	0.6	11	0.6	No effect.
0.6	5	0.8	Do.	0.5	12	0.5	Do.
0.6	5	1.0	Do.	0.6	13	0.7	Do
0.6	4	0.7	No effect.	0.5	17	0.5	Do.
0.6	5	0.6	Do.	0.5	33	0.6	Do.
0.5	5	0.5	Do.	0.2	33	0.6	Do.

Eleven mice were injected with venom on two separate occasions. One lot of six mice was given the second injection 14 days after the first; another lot of five was injected a second time on the twentieth day after the first injection. None of the mice showed any symptoms after the second injection. In the table following the amount of venom injected and the time interval between the two injections are given:

Experiments in anaphylaxis (mice).

First injection.	Time elapsed.	Second injection.	Result.	First injection.	Time elapsed.	Second. injection.	Result.
mg.	*days.*	*mg.*		*mg.*	*days.*	*mg.*	
0.05	14	0.03	No effect.	0.05	20	0.05	No effect.
0.05	14	0.03	Do.	0.05	20	0.05	Do.
0.05	14	0.03	Do.	0.05	20	0.05	Do.
0.05	14	0.05	Do.	0.05	20	0.05	Do.
0.05	14	0.05	Do.	0.05	20	0.05	Do.
0.05	14	0.05	Do.				

Two rabbits were used in these experiments, the time interval between the first and second injection being 36 days and 44 days, respectively. Although both animals received quite large doses of venom at the second injection, neither of them showed any symptoms as a result of the injection.

Experiments in anaphylaxis (rabbits).

Weight.	First injection.	Time elapsed.	Second injection.	Result.
gms.	*mg.*	*days.*	*mg.*	
1300.....	6	44	12	No effect.
1900.....	15	36	12	Do.

In all the experiments (with pigeons, mice, and rabbits), both the first and the second injections were given subcutaneously. It is quite evident that the injection of heloderma venom into an animal does not noticeably increase the susceptibility of the animal to a second dose of venom.

The venom of *Heloderma*, therefore, does not cause typical general anaphylaxis.

III.

BACTERIOLOGY OF THE SALIVA OF HELODERMA SUSPECTUM.

By D. Rivas.

BACTERIOLOGY OF THE SALIVA OF HELODERMA SUSPECTUM.

By D. Rivas.

In the course of investigations into the toxic action of the poison of *Heloderma suspectum*, it was necessary to compare the effect of fresh and heated saliva of these reptiles. It had been found* that if tested on guinea-pigs the fresh venom caused, besides the typical symptoms common to both the heated and fresh venom, certain inflammatory reactions which were absent in those guinea-pigs which had been injected with heated venom. It was of interest, therefore, to determine which organism might be responsible for the inflammatory lesions found in guinea-pigs after injection of fresh venom, and with this point in view experiments were carried out to isolate the organism responsible and to determine whether the organism was present frequently in the saliva of these reptiles.

A guinea-pig which had received an intraperitoneal injection of fresh venom and had been dead for about 3 hours was brought to me for bacteriological examination.

Autopsy.—Point of inoculation slightly swollen and edematous; slight peritoneal exudate, but no congestion. Spleen enlarged; other organs normal.

Microscopical examination.—Smears made from point of inoculation, peritoneal exudate, blood, and organs showed, among other bacteria, a thin Gram negative bacillus which in pure cultures corresponded to the morphological and biological features of *Bacillus coli communis;* it fermented dextrose, produced indol, coagulated milk, did not liquefy gelatine, was negative to test No. 1 and test No 3, and positive to test No. 2 (D. Rivas, Journal of Infectious Diseases, vol. 4, November 1907, p. 641, and Journal of Medical Research, March 1908.)

After obtaining similar results with a number of other guinea-pigs which directly after death presented the same post-mortem appearance as the first animal, experiments were made to determine the virulence of the cultures isolated. A number of guinea-pigs were used for the experiment; some were injected subcutaneously and others intraperitoneally with a small amount of the scraping of a 24-hour agar culture dissolved in sterile salt solution. All injected guinea-pigs died, some a few days after the inoculation and others on the day of the injection, and in one instance the culture was found to be so virulent that the guinea-pig died 3 hours after the intraperitoneal injection. Bacteriological examination of the point of inoculation, blood, and organs of the dead guinea-pigs revealed in each case the presence of the bacillus used for injection.

Cf. the communication by E. Cooke and Leo Loeb.

These results suggested a direct bacteriological examination of the saliva of the *Heloderma suspectum* from which the venom had been obtained. The saliva collected in a sterile pipette from the mouth of the animal was plated, and the isolated colonies studied. Besides many other micro-organisms, *B. coli communis* was found to be abundantly present in the saliva.

The fact that such a virulent strain of *B. coli communis*, able to produce death in so short a time as 3 hours after the injection, was found in the saliva, seems to me to be worthy of special consideration, not merely as a contribution to the virulency of *B. coli communis*, but more especially as indicating a possible source of error in the study of the fresh venom of poisonous reptiles.

In our investigations, besides *B. coli*, *B. pyocyaneus*, and other pathogenic and pyogenic bacteria were found, but attention was given to *B. coli* only, because in this case this organism was found to be more virulent than the others with which it was associated in the saliva.

In a further series, the frequency of the presence of *B. coli* in the saliva of this reptile was investigated. The saliva of ten individuals of this species was examined, in five of which *B. coli* was isolated after a single examination, and it is probable that on repeating the examination of the negative cases, this organism (*B. coli communis*) would have been found in most, if not in all, of the ten helodermas.

IV.

INFLUENCE OF HELODERMA VENOM UPON BLOOD-PRESSURE, DIURESIS, PERITONEAL TRANSUDATE, AND INTESTINAL FLUID.

By Moyer S. Fleisher.

INFLUENCE OF HELODERMA VENOM UPON BLOOD-PRESSURE, DIURESIS, PERITONEAL TRANSUDATE, AND INTESTINAL FLUID.

By Moyer S. Fleisher.

Van Denburgh and Wight,* studying some of the physiological actions of the venom of *Heloderma suspectum*, came to the conclusion that the lethal effect of the venom was due to its influence on respiration. The animals injected with venom died as a result of respiratory failure; at the same time the blood-pressure was lowered. In order to investigate further the influence of the venom on blood-pressure we injected into rabbits large quantities of 0.85 per cent sodium-chloride solution in which venom had been dissolved. We were thus also able to study the effect of venom on the secretion of urine and the transudation of fluid into the peritoneal cavity or into the intestines, as well as the relation existing between the blood-pressure and the secretion of urine.

The 0.85 per cent sodium-chloride solution was allowed to flow into the jugular vein of the rabbit at the rate of 4 c.c. of fluid per minute. By means of a hot-water bath the infused fluid was maintained at a temperature of 39° C. Quantities of fluid varying between 600 and 700 c.c. were infused into the rabbits.

EXPERIMENTS WITH NON-NEPHRECTOMIZED RABBITS.

In a first series of experiments, rabbits with intact kidneys were infused with the sodium-chloride solution, to which venom had been added in the proportion of 20 or 30 mg. to every 800 c.c. of the sodium-chloride solution. Quantities of urine secreted during the inflow of every 50 or 100 c.c. of the NaCl solution were noted. At the end of the experiment, after the inflow of fluid had been stopped, the animal was killed and the quantity of fluid in the peritoneal cavity and small intestines was measured. By means of a mercury manometer connected with the carotid artery of the rabbit, the arterial blood-pressure was measured during the time of the infusion.

Immediately after the infusion of the venom-sodium-chloride solution was started the blood-pressure of the rabbit became rapidly lower; after 20 to 50 c.c. had been infused, a fall of 10 to 40 mm. of mercury could be observed; in our 5 cases the average fall of blood-pressure was 24 mm. After the first rapid fall the blood-pressure remained either approximately stationary or gradually decreased slightly. After 600 or 700 c.c. of the venom-sodium-chloride mixture had been infused the arterial blood-pressure was usually between 80 and 60 mm. of mercury (figs. 15 and 16). In only one of these experiments, toward the end of the infusion, the blood-pressure fell below 50 mm. In this experiment, the only one in which 30 mg. of venom was used, the animal died as a

*Van Denburgh and Wight, Trans. Amer. Phil. Soc., 1898, xix, 199.

result of the fall of blood-pressure (see fig. 17). In all other experiments the animals were alive at the end of the experiment.

During the time of infusion a large quantity of urine, an average of 590 c.c. for 1,000 c.c. of infused fluid, was secreted.

In no case was any ascitic fluid found at the expiration of the experiments, notwithstanding the fact that an average of approximately 300 c.c. of fluid was retained within the bodies of the infused rabbits. Transudation of fluid into the intestinal canal, however, took place. On the average 51 c.c. of intestinal fluid were recovered for every 1,000 c.c. of fluid infused.

TABLE I.—*Non-nephrectomized rabbits infused with venom-sodium-chloride solution.*

Quantity of venom.	Fluid infused.	Urine.	Ascites.	Intestinal fluid.
	c.c.	c.c.		c.c.
20 mg. to 800 c.c.	700	345	0	55
Do	700	360	0	(b)
30 mg. to 800 c.c.	700	365	0	50
20 mg. to 800 c.c.	700	460	0	30
Do	700	505	0	45
Average for every 1,000 c.c. infused		590	0	51
Average for every 1,000 c.c. retained[a]				120

[a]Not measured. [b]Retained fluid equals fluid infused minus amount of fluid eliminated as urine.

Although the quantities of urine eliminated by the animals infused with venom-sodium-chloride solution were relatively very large, yet in a series of control experiments with animals infused with pure sodium-chloride solution even larger quantities of urine were eliminated, namely, on the average 860 c.c. of urine for every 1,000 c.c. of fluid infused. The addition of venom therefore diminished somewhat the secretion of urine.

During the early period of the infusion the quantity of urine secreted is large in spite of the fact that the blood-pressure has fallen quite markedly. It is only after 300 or 400 c.c. have been infused, at a time when the blood-pressure has reached a level which, while fairly constant, is nevertheless below the normal, that the secretion of urine begins gradually to grow less. These experiments do not yet permit us to decide whether the diminished secretion of urine is due to the action of venom in lowering the general arterial pressure or to a local action of venom on the renal vessels or renal cells.

The addition of venom to the infused sodium-chloride solution seems to diminish the transudation of fluid into the peritoneal cavity; in previously published control experiments* on non-nephrectomized rabbits infused with 0.85 per cent sodium-chloride solution, an average of 68 c.c. of fluid for every 1,000 c.c. of fluid which had been retained was found in the peritoneal cavity.

On the other hand, the addition of venom has increased the transudation of fluid into the intestinal cavity. In rabbits infused with pure sodium-chloride solution we found* an average of 53 c.c. in the intestines for every 1,000 c.c. of fluid retained, while an average of 120 c.c. was recovered if venom had been added. Venom causes, therefore, an increase in the elimination of fluid into the intestines.

*Fleisher, Hoyt and Loeb., Jour. Exper. Med., 1909, XI, 291.

According to Porges and Pribram,* and Fleisher and Loeb,† the addition of calcium chloride to an infused sodium-chloride solution causes a lowering of the blood-pressure and a diminution in the secretion of urine. These observations suggested the possibility that the lowered blood-pressure and the lessened elimination of urine in animals injected with venom-sodium-chloride solution were due to some inorganic portion of the dry venom, acting in a manner similar to calcium chloride. Calcium chloride, however, as we have shown previously, diminishes the elimination of urine independently of its action on the general arterial system. We incinerated, therefore, 20 mg. of the dry venom and dissolved the ash in sodium-chloride solution; 20 mg. of venom contain on the average approximately 13 per cent of ash; approximately 2.5 mg. of inorganic material were therefore added to 800 c.c. of salt solution.

When this mixture of venom-ash and sodium-chloride solution was infused into rabbits no fall, but even a slight rise, of blood-pressure was usually seen after the first 50 or 100 c.c. of fluid had been infused. After this rise the pressure either remained at the same level during the course of the infusion or sank very gradually to normal. At no time did the pressure fall as low as it did after the infusion of the dry venom. Thus the infusion of the venom-ash sodium-chloride solution acts in very much the same manner on blood-pressure as the infusion of pure sodium-chloride solution,† and the fall of blood-pressure resulting from the infusion of venom-sodium-chloride solution must consequently be due to the organic portion of the venom (see fig. 18).

The addition of the venom-ash to the sodium-chloride solution does not interfere with the elimination of fluid through the kidneys; for every 1,000 c.c. of fluid infused 780 c.c. of urine were secreted. In these experiments the quantity of fluid present in the intestines at the end of the infusion was so small that it appeared useless to measure it; neither was any ascitic fluid found in these experiments.

TABLE 2.—*Non-nephrectomized rabbits infused with venom-ash sodium-chloride solution.*

Infused.	Urine.	Ascites.	Intestinal fluid.
c.c.	*c.c.*		
600	425	0	..
700	625	0	5
700	540	0	2
700	605	0	3
700	485	0	4

Average for every 1,000 c.c. infused:

	c.c.
Urine	780
Intestinal fluid	5
Average for every 1,000 c.c. retained:	
Intestinal fluid	19

The fact that such a small amount of intestinal fluid and no ascitic fluid were found in these experiments is due to the very small quantities of fluid (an average of 144 c.c.) which were retained within the bodies of the animals. As we have shown previously,‡ when small quantities of fluid are retained within the body, the quantities of peritoneal transudate and intestinal fluid produced

*Porges and Pribram, Arch. für exper. Path. u. Pharm., 1908, LIX, 30.
†Fleisher and Loeb, Jour. Exper. Med., 1909, XI, 641.
‡Fleisher, Hoyt and Loeb, Jour. Exper. Med., 1909, XI, 291.

are very small as compared with the amounts produced when larger quantities of fluid are retained.

The diuresis corresponded to the blood-pressure curve in these experiments. During the infusion of the first 100 or 200 c.c. of venom-ash sodium-chloride solution the secretion of urine increased quite rapidly; and it continued at about the same rate during the whole of the remainder of the period of infusion. In this respect the venom-ash sodium-chloride solution produces approximately the same effect as the pure sodium-chloride solution. The quantity of urine secreted by the animals infused with venom-ash sodium-chloride solution is only slightly less than the amount secreted by the animals infused with sodium-chloride solution, but is distinctly larger than the amount secreted by animals infused with venom-sodium-chloride solution.

The venom-ash exerts no distinct influence on either blood-pressure or secretion of urine, and the changes observed must be due to organic substances of the venom.

In order to determine whether the lessened secretion of urine in the experiments in which venom was added to the infused sodium-chloride solution was due to the lowering of the arterial pressure, we carried out some experiments in which we added adrenalin to the infused fluid. Adrenalin, when infused intravenously into animals, causes a rise of blood-pressure, and this rise is maintained during the whole period of the infusion; at the same time, adrenalin increases the diuresis.*

In one experiment we added 1.5 c.c. of adrenalin to the 800 c.c. of sodium-chloride solution which already contained 20 mg. of venom. In spite of the presence of the adrenalin the infusion of the solution containing venom caused in this case a very marked fall of the blood-pressure during the infusion of the first 100 c.c. of fluid. Also in the later stages, after 400 or 500 c.c. had been infused, the usual gradual fall of pressure was observed. In this experiment adrenalin had little or no effect in preventing the action of venom on the blood-pressure; neither had the diuresis curve been affected by the addition of adrenalin, for we noted the early rise in the curve and the later fall coincident with the fall in blood-pressure (see fig. 19).

Three other experiments were made in a somewhat different manner. The infusion was started with the usual venom-sodium-chloride solution (20 mg. of venom to 850 c.c. of sodium-chloride solution), and after either 150 or 200 c.c. of this mixture had been infused the adrenalin was added. In each of two cases 1 c.c. of adrenalin (making thus approximately a 1 to 600,000 solution), and in the third experiment 6 c.c. of adrenalin (making a 1 to 100,000 solution) were added.

In all these experiments the usual marked fall of blood-pressure was observed when the first 50 or 100 c.c. of venom-sodium-chloride solution were infused. After the addition of the adrenalin (1 to 600,000) the blood-pressure rose slightly, and it continued at approximately the same level until the con-

*Fleisher and Loeb, Jour. Exper. Med., 1909, xi, 480.

clusion of the experiment. The addition of small quantities of adrenalin causes, therefore, a slight rise in the blood-pressure, and prevents, or at least delays, to some extent, the usual fall after 500 or 600 c.c. of venom-sodium-chloride solution has been infused (see figs. 20 and 21).

In the experiments in which the 1 to 600,000 adrenalin solution was used the diuresis increased quite markedly after the infusion of the adrenalin, and the secretion of urine reaches a relatively high level, when compared with the secretion at a similar period, during the infusion of the venom-sodium-chloride solution. This was true in two of the experiments at least. In one experiment no decrease either of diuresis or blood-pressure was observed in the late stages of the infusion, whereas in the other experiment a slight decrease in the secretion of urine is noted simultaneously with a slight fall in the blood-pressure.

In a third experiment,* in which we infused a 1 to 100,000 adrenalin solution during the second period of the experiment, the rise of blood-pressure following the infusion of the adrenalin solution was more pronounced than in the other two, and at the same time the secretion of urine was very markedly increased after addition of the adrenalin (see fig. 24).

From these experiments we may conclude that adrenalin counteracts to a small extent the blood-pressure lowering action of the venom. In view of the fact that in one experiment the blood-pressure began again to fall even after the addition of adrenalin, it is probable that this influence of adrenalin would eventually be overcome by the depressing action of the venom. We further note that simultaneously with the rise of blood-pressure following the addition of adrenalin, the diuresis is also increased; it is therefore probable that the diminution of diuresis resulting from the addition of venom to the infused fluid is due not to a specific action of the venom on the kidneys, but to the lowering of the arterial pressure.

The average amount of urine secreted per 1,000 c.c. infused was greater in the experiments in which adrenalin was added to the venom-sodium-chloride than in the experiments in which venom-sodium-chloride solutions alone were infused. In these experiments, as well as in the former experiments with venom but without the addition of adrenalin, the quantity of intestinal fluid was increased, while the ascitic fluid was absent.

TABLE 3.—*Non-nephrectomized rabbits infused with venom-sodium-chloride solutions to which adrenolin was added.*

Infused.	Urine.	Ascites.	Intestinal fluid.
c.c.	c.c.		c.c.
500	405	0	..
700	385	0	40
700	490	0	26
700	550	0	25

Average for every 1,000 c.c. infused:

	c.c.
Urine	700
Intestinal fluid	91

*In this experiment a large quantity of venom was injected intravenously some time after the infusion of adrenalin was started, but at a time when the influence of the addition of adrenalin had been clearly demonstrated.

Furthermore, we have in a few experiments injected in one dose a large quantity of venom intravenously, while the sodium-chloride solutions were being infused at the usual rate. The rabbit was infused in the usual manner with a 0. 85 per cent sodium-chloride solution; after several hundred cubic centimeters of this solution had been infused, when the blood-pressure was high and the diuresis markedly increased, the infusion was stopped for a few moments while the venom solution was injected directly into the jugular vein. The infusion was then continued as before, with 0.85 per cent sodium-chloride solution. In one experiment the rabbit was infused with venom-adrenalin-sodium-chloride solution instead of sodium-chloride solution, both before and after the injection of venom was given.

As stated above, the injection was given at a time when the blood-pressure was either higher than usual or nearly normal and when the diuresis was marked. Immediately following the injection we observed a marked fall of blood-pressure, which was 24 mm., 62 mm., and 89 mm. of mercury in the three cases respectively. Following this very marked lowering of the blood-pressure it rose again 10 to 18 mm. during the inflow of the next 100 c.c. of fluid. When a pure sodium-chloride solution was infused both before and after the injection of the venom, the blood-pressure continued from there on at about the same level (still quite markedly below the normal) until the end of the experiment (see figs. 22 and 23). When adrenalin-venom-sodium-chloride solution was infused both before and after the sudden injection of the venom, the pressure fell again after the first rebound but later rose very gradually (see fig. 24). In this latter case it is probable that exhaustion prevented the blood-pressure from rising as much as it did in cases in which pure sodium-chloride solution was infused.

The diuresis curve shows a very marked fall simultaneously with the fall of blood-pressure. In experiments in which a pure sodium-chloride solution was used in the period directly following the rapid injection of the venom, only 6 to 10 c.c. of urine were secreted during the infusion of the first 100 c.c. of fluid. As the blood-pressure now became gradually higher, the secretion of urine also increased, 20 to 60 c.c. of urine being eliminated, while 100 c.c. of fluid were being infused. In the experiment in which venom-adrenalin-sodium-chloride solution was infused the decrease in the diuresis, as well as in the blood-pressure, was not as marked as in the experiments in which pure sodium-chloride solution was infused. The presence of the adrenalin in the infused fluid diminished probably to some extent the fall of blood-pressure and decrease in diuresis.

The rapid injection of venom produces a fall of blood-pressure much more marked than after the continuous infusion of the dilute solution of venom. Accompanying this fall of blood-pressure, the secretion of urine is very markedly diminished. As the pressure gradually rises the diuresis becomes again more pronounced.

It is of interest to note that the quantity of venom injected in two cases, namely, 12 mg., would be a fatal dose if injected intravenously into a rabbit under normal conditions, while the 36 mg. which were injected in the other

experiment is considerably larger than the lethal dose. Yet none of the animals succumbed to the injection! It appears, therefore, that the venom is either very rapidly destroyed within the bodies of the animals or prevented from combining with the central nervous system, either as a result of being very rapidly eliminated from the body or in some other way. It seems most probable that the failure of the venom to cause death under these conditions is not due to the destruction but perhaps to the elimination of the venom, since in the normal uninfused animals the same possibilities for the breaking-down of the venom exist, whereas the possibilities for the elimination of the venom are not so good as in the infused rabbits.

These experiments make it highly probable that the venom acts only indirectly on the diuresis by influencing the general arterial blood-pressure and that it does not exert a direct influence on renal cells or vessels. With the fall of blood-pressure the secretion of urine diminishes, while with the increasing pressure the secretion of urine also increases.

EXPERIMENTS WITH NEPHRECTOMIZED RABBITS.

In further experiments we infused venom-sodium-chloride solution into rabbits whose kidneys had previously been removed. Under such conditions all the infused fluid was retained within the body of the animal, and we were able to determine more exactly the influence of the addition of venom to the sodium-chloride solution on the production of peritoneal transudate and intestinal fluid. We carried out seven experiments. In one 600, in four 700, and in two 800 c.c. were infused. In all these experiments 20 mg. of venom had been added to 800 c.c. of sodium-chloride solution. An average of 104 c.c. of peritoneal transudate was produced for every 1,000 c.c. of fluid infused. In control experiments, carried out some time ago,* and in which pure sodium-chloride solution was infused into rabbits, 109 c.c. of peritoneal transudate were produced for every 1,000 c.c. of fluid infused. The addition of venom to the sodium-chloride solution has, therefore, little or no effect on the production of peritoneal transudate.

Before the infusion was started, and at the time the kidneys were removed, the small intestines were clamped at both the upper and lower ends, and at the conclusion of the experiment the fluid contained in them was measured in each case. An average of 130 c.c. of fluid was found in the intestines for every 1,000 c.c. of fluid infused. In previous experiments in which sodium-chloride solution alone was infused,* we had found an average of 94 c.c. of fluid was collected from the intestines for every 1,000 c.c. infused. Thus the addition of venom to the infused fluid had increased the transudation of fluid into the intestines, a finding in agreement with the results of similar experiments in non-nephrectomized animals and also with the autopsy findings of animals that had died after a single injection of venom.

*Fleisher, Hoyt and Loeb, Jour. Exper. Med., 1909, xi, 291.

TABLE 4.—*Nephrectomized rabbits infused with venom-sodium-chloride solution.*

Infused.	Ascites.	Intestinal fluid.
c.c.		c.c.
600	45	55
700	68	84
700	69	70
700	65	110
700	82	106
800	120	135
800	70	90

For every 1,000 c.c. infused:

Ascites..... 104
Intestinal fluid..... 130 c.c.

Thus, in spite of the fact that the addition of venom causes a lowering of the blood-pressure, the transudation of fluid into the peritoneal cavity is not diminished and the transudation of fluid into the intestines is increased, if the venom is added to the NaCl solution before the infusion.

CONCLUSIONS.

(1) The addition of venom to sodium-chloride solution which is infused intravenously into rabbits causes a marked and rapid fall of blood-pressure. If the infusion is extended over some time, the pressure continues to fall gradually.

(2) This fall of blood-pressure is due to the organic constituents of venom.

(3) The addition of adrenalin to the venom-sodium-chloride solution counteracts only to a slight extent the blood-pressure-lowering effect of the venom, and it is probable that eventually if the infusion were continued over a sufficiently long period of time, the influence of the venom would overcome the action of the adrenalin.

(4) The addition of venom to the infused sodium-chloride solution diminishes the secretion of urine. Since the diuresis curve varies with the blood-pressure curve it is probable that venom does not cause a decrease in diuresis by a direct action on the kidney, but through its influence on the general arterial blood-pressure.

(5) The rapid intravenous injection of a large dose of venom into a rabbit which is being infused with sodium-chloride solution causes a marked and very rapid fall of blood-pressure which is accompanied by a very marked decrease in the secretion of urine. When the infusion is continued the blood-pressure rises again and the diuresis increases.

(6) Animals which are being infused with sodium-chloride solution will resist the intravenous injection of a quantity of venom which under normal conditions would be lethal. This resistance is probably due to the rapid elimination of the venom.

(7) The addition of venom to the sodium-chloride solution appears to have no influence on the production of ascitic fluid, while it causes an increase in the elimination of fluid into the intestinal cavity, notwithstanding the owered blood-pressure.

PROTOCOLS.

EXPERIMENT I.

[Rabbit, weight 2,200 gms. Infused with sodium-chloride solution plus venom (20 mg. to 800 c.c.).]

Amount infused.	Blood pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	120	...
50	90	...
100	86	50
200	80	80
300	80	70
400	72	60
500	72	70
600	72	20
700	72	35
Total		385

EXPERIMENT II.

[Rabbit, weight 1,900 gms. Infused with sodium-chloride solution plus venom (20 mg. to 800 c.c.).]

Amount infused.	Blood pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	190	...
20	60	...
100	56	5
200	58	20
300	60	65
400	62	80
500	56	80
600	56	55
700	54	55
Total		360

EXPERIMENT III.

[Rabbit, weight 1,760 gms. Infused with sodium-chloride solution plus venom (30 mg. to 800 c.c.).]

Amount infused.	Blood-pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	128	...
10	102	...
50	106	...
100	92	55
200	82	60
300	88	55
400	84	65
500	70	50
600	68	50
700	50	30
Total		365

EXPERIMENT IV.

[Rabbit, weight 1,700 gms Infused with sodium-chloride solution plus incinerated venom (20 mg. to 800 c.c.).]

Amount infused.	Blood-pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	110	...
100	110	83
200	84	100
300	90	75
400	88	90
500	86	95
600	86	90
700	86	92
Total		625

EXPERIMENT V.

[Rabbit, weight 1,700 gms. Infused with sodium-chloride solution and incinerated venom (20 mg to 800 c.c.).]

Amount infused.	Blood-pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	100	...
20	120	...
100	110	63
200	100	82
300	110	80
400	114	75
500	114	70
600	116	85
700	116	85
Total		540

EXPERIMENT VI.

[Rabbit, weight 1,700 gms. Infused with sodium-chloride solution and incinerated venom (30 mg. to 800 c.c.).]

Amount infused.	Blood-pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	80	...
20	86	...
50	100	...
100	90	41
200	96	104
300	84	95
400	96	85
500	100	85
600	104	90
700	106	105
Total		605

EXPERIMENT VII.

[Rabbit, weight 1,700 gms. Infused with sodium-chloride solution plus incinerated venom (25 mg. to 800 c.c.).]

Amount infused.	Blood-pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	120	...
20	148	...
100	150	20
200	150	45
300	154	65
400	150	80
500	150	90
600	150	100
700	150	85
Total		485

EXPERIMENT VIII.

[Rabbit, weight 1,700 gms. Infused with sodium-chloride solution plus incinerated venom (25 gm. to 800 c.c.).]

Amount infused.	Blood-pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	110	...
20	130	...
100	126	15
200	120	60
300	122	75
400	124	95
500	106	90
600	110	90
Total		425

EXPERIMENT IX.

[Rabbit, weight 2,400 gms. Infused with sodium-chloride solution plus venom (20 mg. to 800 c.c.); at 200 c.c. added 1 c.c. adrenalin.]

Amount infused.	Blood-pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	96	...
10	112	...
20	84	...
50	80	...
100	70	40
*200	72	50
300	76	80
400	76	95
500	80	95
600	82	90
700	82	100
Total		550

*Added adrenalin.

Experiment X.

[Rabbit, weight 1,800 gms. Infused with sodium-chloride solution plus adrenalin (1.5 c.c. to 1,000 c.c.) plus venom 20 mg. to 800 c.c.).]

Amount infused.	Blood-pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	140	...
10	112	...
30	100	...
100	92	25
200	86	75
300	90	100
400	90	85
500	82	85
600	70	65
700	64	50
Total	.....	485

Experiment XI.

[Rabbit, weight 1,900 gms. Infused with sodium-chloride solution plus venom (20 mg. to 800 c.c.); at 150 c.c. added 1 c.c. adrenalin.]

Amount infused.	Blood-pressure.	Urine.
c.c.	*m.m.*	*c.c.*
0	84	...
30	96	...
50	76	...
100	70	15
130	66	...
*150	72	15
200	72	25
300	70	50
400	74	75
500	72	80
600	66	65
700	62	60
Total	.....	385

*Added ndrenalin.

Experiment XII.

[Rabbit, weight 1,700 gms. Infused with sodium-chloride solution plus venom (20 mg. to 800 c.c.); at 150 c.c. added 6 c.c. adrenalin; at 500 c.c. injected 12 mg. venom intravenously.]

Amount infused.	Blood-pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	100	...
10	84	...
20	72	...
50	76	...
100	68	52
150	70	23
200	74	30
300	82	88
400	82	112
500	82	102
Injected 12 mg. venom and started infusion again.		
c.c.	*mm.*	*c.c.*
510	52	...
520	60	15
540	64	...
550	58	...
600	56	36
650	50	...
700	60	20
	58	30
Total	.....	508

Experiment XIII.

[Rabbit, weight 1,900 gms. Infused with sodium-chloride solution; nt 300 c.c. injected 12 mg. venom intravenously.]

Amount infused.	Blood-pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	124	...
80	128	...
100	122	53
200	102	120
300	120	110
Injected 12 mg. venom and continued infusion again.		
c.c.	*mm.*	*c.c.*
5	60	...
20	52	...
40	50	...
90	54	...
100	58	(*)
200	60	15
300	62	42
	64	60
Total	.....	400

*Urine begins.

Experiment XIV.

[Rabbit, weight 1,800 gms. Infused with sodium-chloride solution; at 300 c.c. injected 36 mg. venom intravenously.]

Amount infused.	Blood-pressure.	Urine.
c.c.	*mm.*	*c.c.*
0	100	...
20	120	...
100	126	35
200	114	105
300	114	110
Injected 36 mg. venom and continued infusion.		
c.c.	*mm.*	*c.c.*
	60	...
5	44	...
20	36	...
30	44	...
60	54	...
100	52	6
200	52	10
300	54	15
400	52	20
Total	.....	301

In the following charts the straight line equals blood-pressure, the dotted line equals serum.

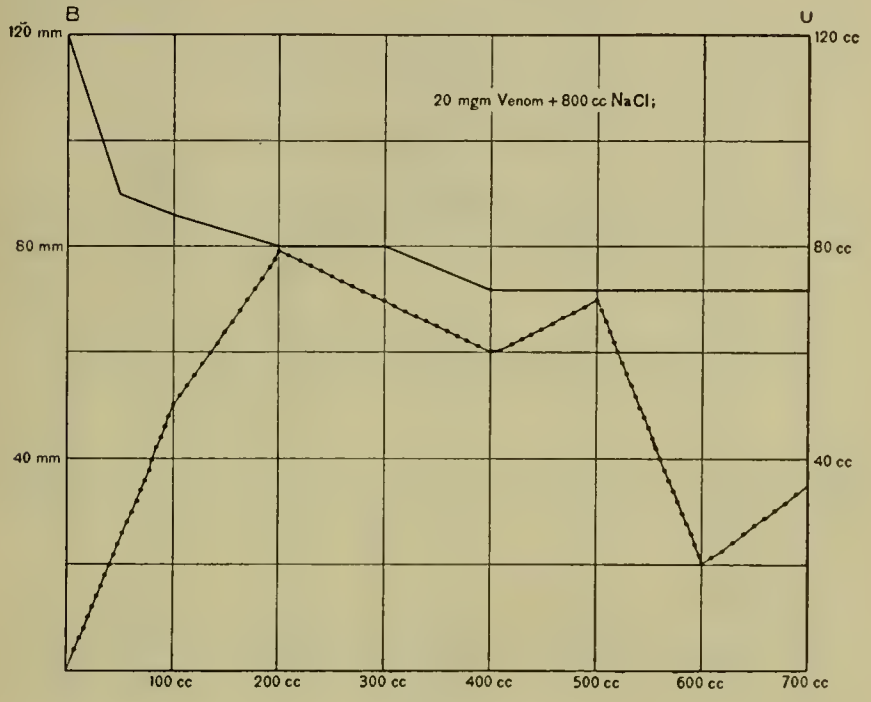

Fig. 15.

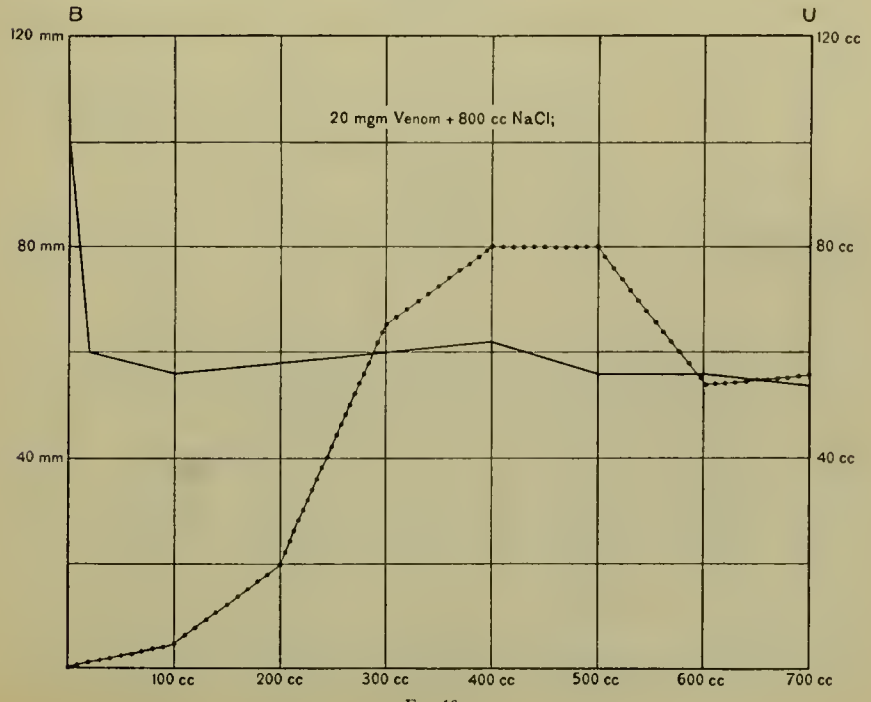

Fig. 16.

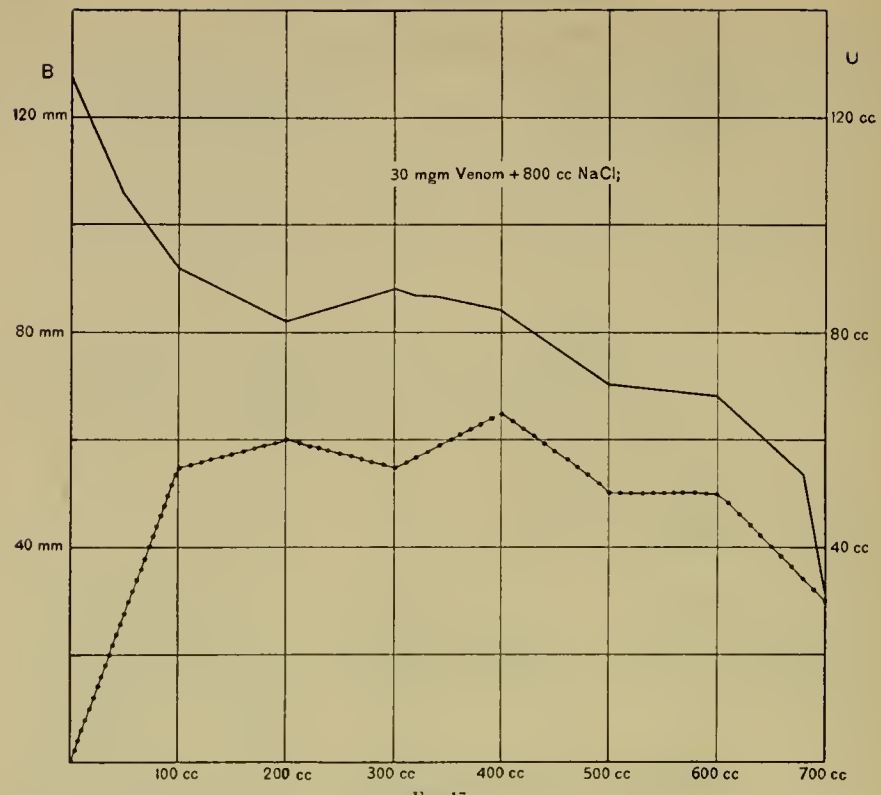
B
U
120 mm
120 cc
30 mgm Venom + 800 cc NaCl;
80 mm
80 cc
40 mm
40 cc
100 cc
200 cc
300 cc
400 cc
500 cc
600 cc
700 cc

FIG. 17.

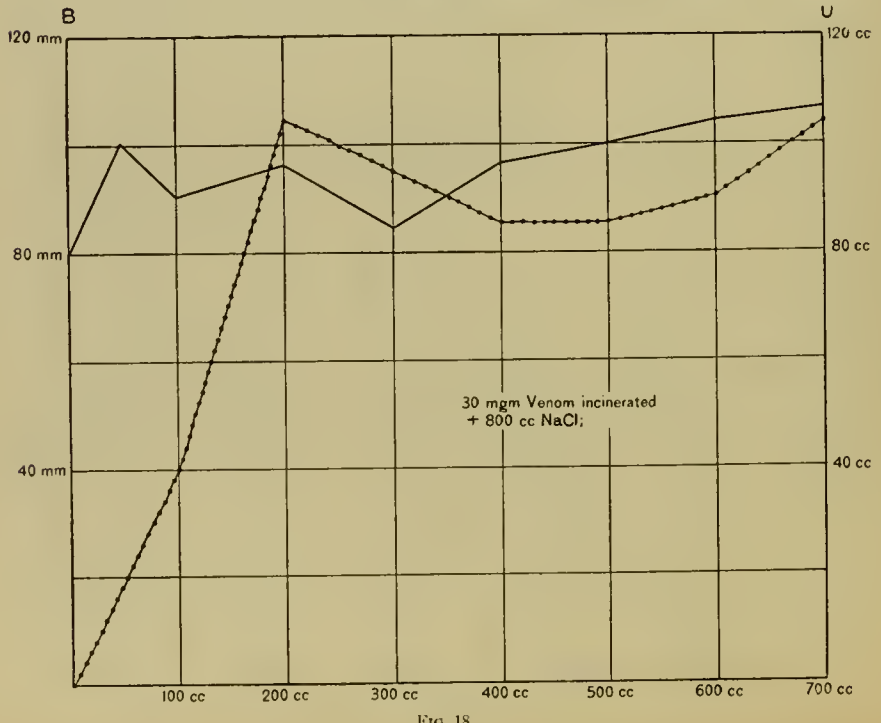
B
U
120 mm
120 cc
80 mm
80 cc
30 mgm Venom incinerated
+ 800 cc NaCl;
40 mm
40 cc
100 cc
200 cc
300 cc
400 cc
500 cc
600 cc
700 cc

FIG. 18.

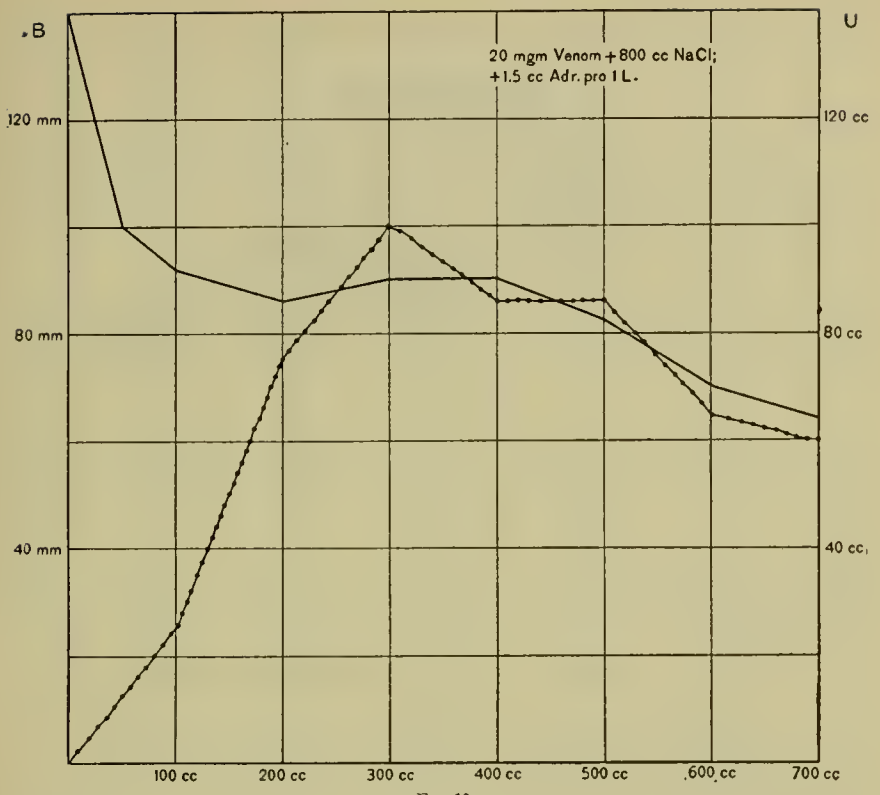
B
U
20 mgm Venom + 800 cc NaCl;
+1.5 cc Adr. pro 1 L.
120 mm
80 mm
40 mm
120 cc
80 cc
40 cc
100 cc
200 cc
300 cc
400 cc
500 cc
600 cc
700 cc

Fig. 19.

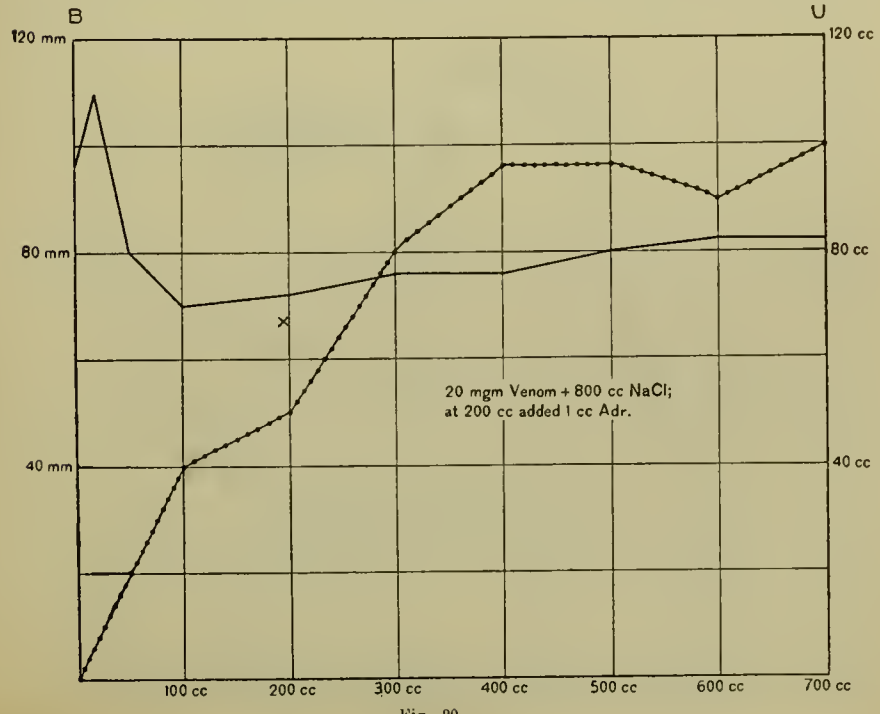
B
U
120 mm
80 mm
40 mm
120 cc
80 cc
40 cc
20 mgm Venom + 800 cc NaCl;
at 200 cc added 1 cc Adr.
100 cc
200 cc
300 cc
400 cc
500 cc
600 cc
700 cc

Fig. 20.

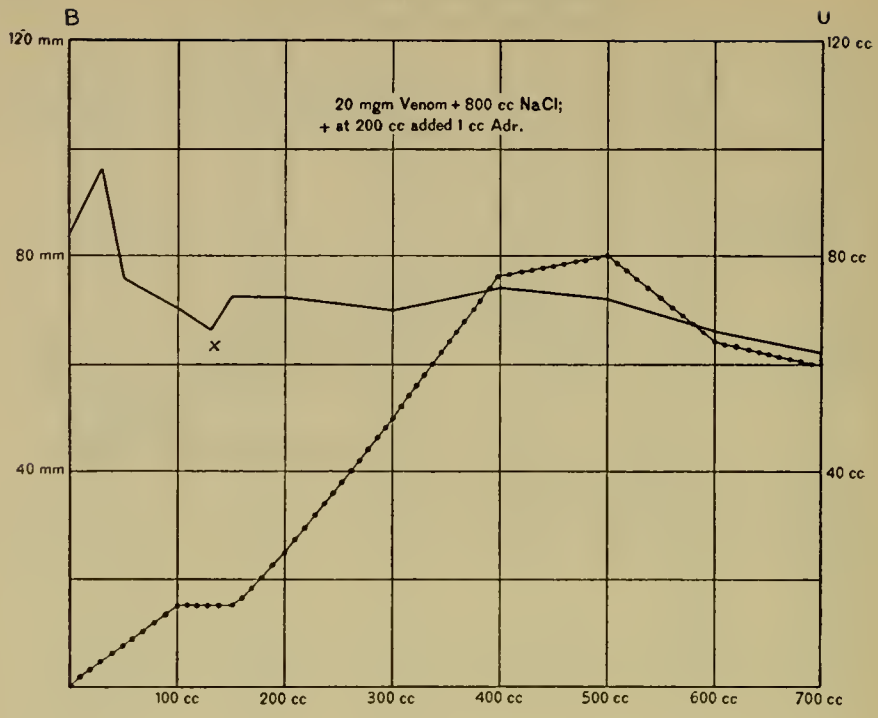
B
U
120 mm
120 cc
20 mgm Venom + 800 cc NaCl;
+ at 200 cc added 1 cc Adr.
80 mm
80 cc
X
40 mm
40 cc
100 cc
200 cc
300 cc
400 cc
500 cc
600 cc
700 cc

FIG. 21.

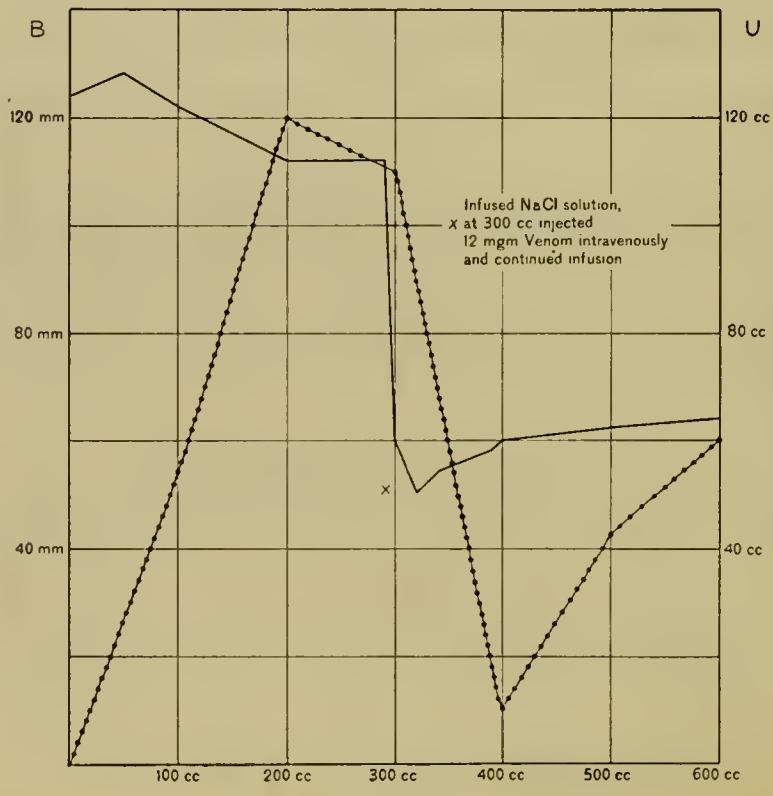
B
U
120 mm
120 cc
Infused NaCl solution,
X at 300 cc injected
12 mgm Venom intravenously
and continued infusion
80 mm
80 cc
X
40 mm
40 cc
100 cc
200 cc
300 cc
400 cc
500 cc
600 cc

FIG. 22.

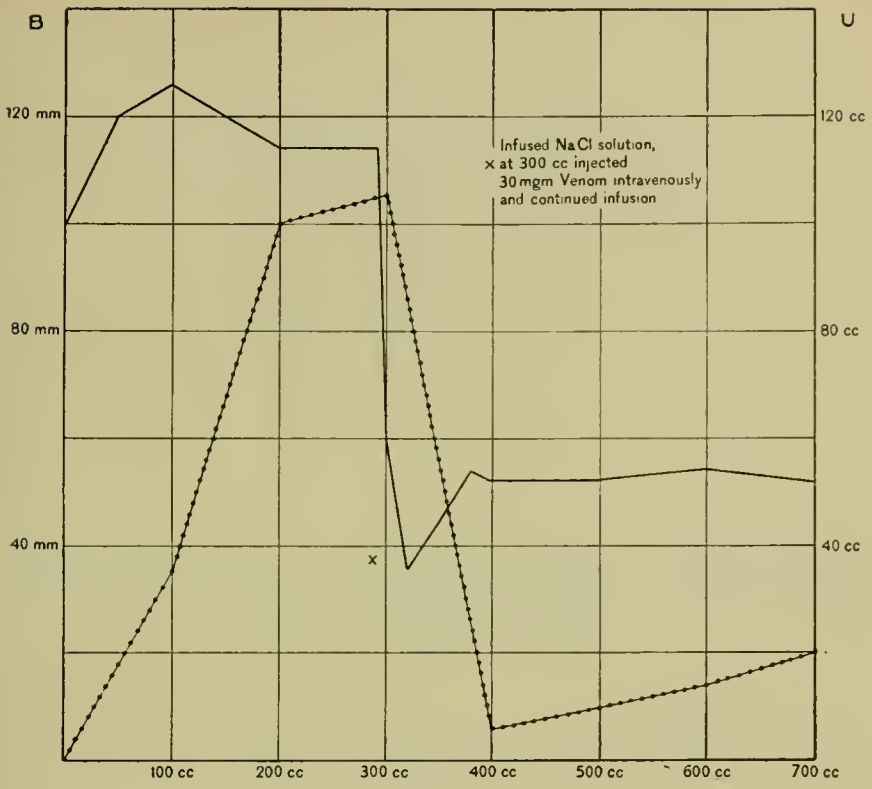
B
U
120 mm
80 mm
40 mm
120 cc
80 cc
40 cc
Infused NaCl solution,
× at 300 cc injected
30 mgm Venom intravenously
and continued infusion
100 cc
200 cc
300 cc
400 cc
500 cc
600 cc
700 cc

FIG. 23.

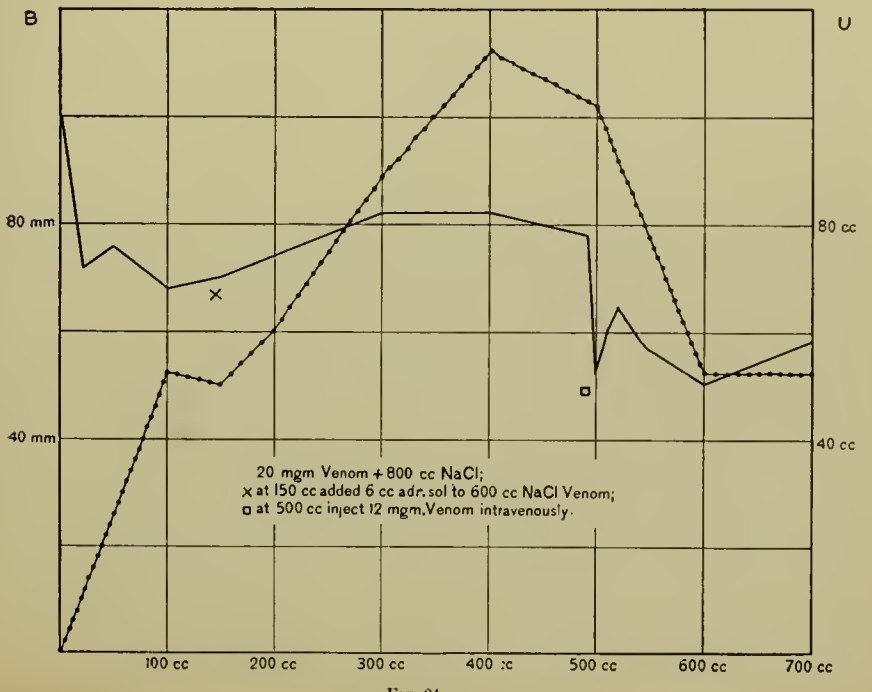
B
U
80 mm
40 mm
80 cc
40 cc
20 mgm Venom + 800 cc NaCl;
× at 150 cc added 6 cc adr. sol to 600 cc NaCl Venom;
□ at 500 cc inject 12 mgm. Venom intravenously.
100 cc
200 cc
300 cc
400 cc
500 cc
600 cc
700 cc

FIG. 24.

V.

EFFECT OF THE VENOM OF HELODERMA SUSPECTUM ON THE ISOLATED HEART.

By Thomas Stotesbury Githens.

EFFECT OF THE VENOM OF HELODERMA SUSPECTUM ON THE ISOLATED HEART.

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In order to determine whether or not the influence of the venom of the Gila monster on blood-pressure was dependent upon a paralyzing action on the cardiac muscle, and also to determine the effects of this venom on muscle-tissue *in vitro*, two series of experiments were carried out, one on strips of the cardiac muscle of the turtle, the other on the isolated heart of the frog. The results seem to show that the direct action of the venom on the heart-muscle plays only a small part in the toxic action exhibited when it is injected into the living animal. The venom seems to exert a slight toxic action on the isolated heart, but does not exert a noticeable cytolytic influence upon heart-muscle tissue *in vitro*. These results agree with physiological tests which show that the venom of the Gila monster, like some snake venoms, acts mainly on the respiratory center, and that the heart continues to beat after respiration has ceased. The strips of muscle which were exposed to the action of the venom usually ceased to beat somewhat sooner than the controls, but not enough to indicate a very marked toxic action on the heart. The isolated heart, transfused with solutions of venom, was markedly affected only by very high concentrations.

ACTION ON STRIPS OF TURTLE HEART.

The technique of this series was as follows: The heart was removed and the ventricle cut into four longitudinal strips, extending from the base to the apex, the auricle being used as a fifth strip. No difference was found in the behavior of the auricle, except that the latent period was somewhat shorter. If such strips are placed in 0.7 per cent NaCl solution, they will begin to contract rhythmically in from 30 minutes to 2 hours, these rhythmic contractions continuing about the same length of time and then gradually ceasing. If small amounts of calcium and potassium are now added to the NaCl solution, or if the strips are transferred to Ringer's solution, rhythmic contractions will again begin. If the contractions cease they may often be induced to recommence by transferring back to 0.7 per cent NaCl solution for a time and returning once more to the Ringer's solution. The length of time during which contractions continue is more or less irregular, even under the best conditions. In our experiments the controls lived from 9 to 36 hours.

In some experiments the venom was dissolved in 0.7 per cent NaCl solution and the strips placed in this on their removal from the body, and when the

contractions had almost ceased calcium and potassium chlorides were added. In others, the venom was dissolved in Ringer's solution and the strips transferred to this at the same period. In some of the experiments the strips were allowed to remain in the venom solution until they ceased to contract; in others they were left in the venom only part of the time. These differences may be seen by referring to the protocols. The amount of venom varied in the different experiments between 0.1 and 0.01 per cent.

In order to determine the effect of possible inorganic substances, some tests were made with a solution of the ash from incinerated venom. This was found to have no influence on the heart. Certain lots of venom were heated to 80° C. for an hour and then kept at 37° C. for 3 days. This heating did not seem to influence the activity. The "heated venom" of the protocols was prepared in this way.

The extreme toxicity shown by sample B (see protocols 7 to 10) was evidently due to some artificial contamination, possibly from the saliva or mouth, and not to the venom itself, as the effect of this venom when injected was not different from that of the others.

PROTOCOLS.

(1) *Venom A.*—Heart of small painted turtle (*Chrysemys picta*) removed and the ventricle cut into five pieces, equal in size. Each strip placed, at 3 p. m., in normal salt solution containing venom as follows: No. 1, 0.07 per cent; No. 2, 0.035 per cent; No. 3, 0.015 per cent; No. 4, 0.005 per cent; No. 5, control. All began irregular contractions which soon ceased. At 5 p. m. all were beating; at 5^h 30^m Nos. 1 and 2 ceased; at 7 p. m. No. 4 ceased; at 9 p. m. Nos. 3 and 5 stopped.

(2) *Venom A.*—Heart of slightly larger painted turtle, treated as before. The auricle in one piece was used as an additional control. Amounts of venom as in 1. Heart removed at 3 p. m. At 6 p. m. Nos. 1, 2, and 3 had ceased to beat. Added 1 to 10,000 calcium chloride to each, which immediately started contractions. At 6^h 30^m No. 1 contracted every 28 seconds; No. 2 every 120 seconds; No. 3 every 12 seconds; No. 4 every 15 seconds; No. 5 every 25 seconds; No. 5*a* every 6 seconds. At 8^h 30^m No. 1 and No. 2 have ceased entirely; No. 3 almost entirely; Nos. 4, 5, and 5*a* still beat strongly. At 9^h 30^m No. 5*a* stopped; at 6 a. m. No. 4 stopped, and soon after No. 5 stopped.

(3) *Venom A.*—Heart of painted turtle. Ventricle cut into four strips; auricle used as No. 2. Placed at 3 p. m. in salt solution containing venom as follows: No. 1, 0.1 per cent; No. 2, 0.05 per cent; No. 3, 0.025 per cent; No. 4, ash of 0.05 per cent; No. 5, control. At 5 p. m. all beating feebly; add to each 0.03 per cent $CaCl_2$ and 0.01 per cent KCl. All begin contractions. At 6 p. m. all still beating, but more feebly. Add 0.01 per cent $CaCl_2$, contractions increase. At 10^h 30^m all have ceased. Transfer to plain 0.85 per cent NaCl solution; only No. 5 beats. At 11^h 30^m transfer to Ringer's solution without venom for the night. The following day at 10 a. m. transfer to plain 0.85 per cent NaCl solution and 5 minutes later to venom; Nos. 1 and 3 contract, No. 1 ceasing soonest.

(4) *Venom A.*—Heart of painted turtle. Ventricle cut in four strips, auricle set as No. 2. Removed at 3 p. m. and placed in salt solution containing venom as follows: No. 1, 0.08 per cent; No. 2, 0.04 per cent; No. 3, 0.02 per

cent; No. 4, 0.01 per cent; No. 5, control. At 4^h 30^m all beating feebly and irregularly. Add to each 1 to 10,000 $CaCl_2$, all beats strengthened. At 6 p. m. all beating feebly. Make all solutions up to 0.03 per cent $CaCl_2$ and 0.02 per cent KCl. At 7 p. m. all have ceased. Transfer to normal NaCl solution for 30 minutes and then back to the old mixture. All begin to beat and continue for several hours. At 8 a. m. transfer all to plain NaCl solution. All but No. 3 begin to beat. At 3 p. m. Nos. 2, 4, and 5 are still beating. At 4 p. m. Nos. 2 and 4 stopped and No. 5 stopped soon after.

(5) *Venom A.*—Venom dissolved in Ringer's solution containing venom as follows: No. 1, 0.05 per cent; No. 2, 0.025 per cent; No. 3, 0.05 per cent, heated; No. 4, 0.025 per cent, heated; No. 5, control. Heart removed at 12 noon and placed in 0.85 per cent NaCl solution. 3 p. m. transfer to venom, all beat, No. 2 very feebly. 4^h 45^m p. m. all still beating; transfer to 0.85 per cent NaCl solution, all beat. 6 p. m. transfer to venom, all beat feebly. 8^h 20^m p. m. all still.

(6) *Venom A.*—To determine whether the slight effect of this venom was due to inorganic constituents, 0.0106 gm. of the venom was incinerated, the ash weighing 0.0012 gm. The heart was removed in the usual manner and placed in normal salt solution containing venom as follows: No. 1, 0.05 per cent venom; No. 2, 0.025 per cent venom; No. 3, ash from 0.05 venom; No. 4, ash from 0.025 per cent venom; No. 5, control. Heart removed at 3 p. m. At 8 p. m. only No. 1 beating, add 0.02 per cent $CaCl_2$ to each. 12 p. m. all beating. 12^h 30^m a. m. No. 2 still, No. 5 very feeble. 7 a. m. all have ceased.

(7) *Venom B.*—Heart removed at 3^h 30^m p. m. and at 6^h 45^m p. m., after latent period, placed in Ringer's solution containing venom as follows: No. 1, 0.04 per cent; No. 2, 0.04 per cent; No. 3, 0.04 per cent, heated; No. 4, 0.04 per cent, heated; No. 5, control. At 8 p. m. all had stopped and on transfer to plain 0.85 per cent NaCl solution only No. 5 beats. This continued several hours; the others never beat again.

(8) *Venom B.*—Heart removed at 1 p. m. and placed in plain 0.85 per cent NaCl solution. At 5 p. m. placed in Ringer's solution containing venom as follows: No. 1, 0.08 per cent; No. 2, 0.04 per cent; No. 3, 0.02 per cent; No. 4, 0.01 per cent; No. 5, control. All stopped in about 3 hours, except control, which continued for over 36 hours.

(9) *Venom B.*—Heart removed at 3 p. m. and placed in normal 0.85 per cent NaCl solution containing venom as follows: No. 1, 0.016 per cent; No. 2, 0.008 per cent; No. 3, 0.004 per cent; Nos. 4 and 5, control. Only the controls started to beat. The strips in the venom were immediately paralyzed and none showed any tendency to recovery on being placed in normal 0.85 per cent NaCl solution or Ringer's solution. They became so soft that they rolled up into a ball by their own weight when the hook on which they were hung was turned over.

(10) *Venom B.*—Heart removed at 3 p. m. and placed in normal NaCl solution containing venom as follows: No. 1, 0.004 per cent; No. 2, 0.002 per cent; No. 3, 0.001 per cent; No. 4, ash from 0.004 per cent; No. 5, control. Only Nos. 4 and 5 started to beat, the others passing into the relaxed condition described in the last experiment.

(11) *Venom C.*—Heart removed at 4 p. m. and the strips placed in NaCl solution containing venom as follows: No. 1, 0.002 per cent; No. 2, 0.001 per cent; No. 3, 0.0005 per cent; No. 4, 0.00025 per cent; No. 5, control. At 7^h 30^m p. m. all were beating very feebly; added to each $CaCl_2$ 3 to 10,000 and KCl 1 to 10,000. All start to beat. At 11^h 30^m p. m. all still beating. At 12 p. m. all have ceased except No. 5, which is still beating, but very slowly and feebly.

ACTION ON THE ISOLATED HEART OF THE FROG.

In this series hearts of leopard frogs (*Rana pipiens*) weighing from 60 to 70 grams were used. They were immersed in Ringer's solution of the composition NaCl 0.6 per cent, $NaHCO_3$ 0.03 per cent, $CaCl_2$ 0.035 per cent, KCl 0.03 per cent, and were transfused by a similar solution in which the venom was dissolved.

The venom solutions varied in strength from 0.01 to 2 per cent. Solutions of less than 0.1 per cent had little if any effect. Solutions of 0.5 and 2 per cent had respectively a moderate and a marked action in reducing the strength of the contractions. Even after the heart had been exposed to the action of such solutions for 15 to 30 minutes, washing out with fresh Ringer's solution restored the strip at once to its former strength, showing that no marked toxic action had been exerted and that no permanent structural change due to cytolysis had been produced.

PROTOCOLS.

Heart No. 1.—Ringer's solution for 6 minutes until normal rhythm was established. Venom 0.01 per cent, renewed every 5 minutes for 24 minutes. Heart stops beating, but on single touch gives a long series of beats of same rate and strength as before the action of the venom. 32 minutes, Ringer; 47 minutes, venom 0.02 per cent; 60 minutes, beats as strong as ever, becoming slower; on touch, series of rapid beats; 70 minutes, Ringer, beats continue slow; 78 minutes, venom 0.1 per cent, beats continue to become slower but show no change in force; 102 minutes, venom 0.5 per cent, beats continue 5 minutes, with no loss in strength.

Heart No. 2.—Ringer's solution to establish rhythm; 13 minutes, venom 0.2 per cent, beats grow weaker, but on renewing venom return to original strength, 22 minutes, no loss of strength; 33 minutes, beats much reduced; Ringer, beats immediately restored to full strength.

Heart No 3.—Ringer's solution for 9 minutes; venom 0.5 per cent; 24 minutes, heart beating in groups but strongly; 26 minutes, heart stops, stimulation causes single beats or short groups; 39 minutes, Ringer, heart begins again in widely separated groups, beats of full strength but apparently some loss of spontaneous impulse.

Heart No. 4.—Ringer, heart beating strongly in groups; 20 minutes, venom 2 per cent, beats immediately, much weaker but no change in rhythm; 32 minutes, Ringer, beats immediately restored to full strength; 42 minutes, venom 2 per cent, beats weaker and grow weaker until 52 minutes when put into Ringer solution, beats immediately restored to full strength, that is, about 1.25 to 1.5 times as high as those under the influence of venom 2 per cent.

VI.

EXPERIMENTAL PRODUCTION OF ACUTE GASTRIC ULCER IN THE GUINEA-PIG.

By M. E. Rehfuss.

EXPERIMENTAL PRODUCTION OF ACUTE GASTRIC ULCER IN THE GUINEA-PIG.*

By M. E. Rehfuss.

It is here proposed to describe somewhat more in detail the results of our experiments and to give a very short résumé of the contributions by some authors bearing on the formation of hemorrhagic erosions and acute gastric ulcer of the stomach. This was the most typical lesion found in guinea-pigs which succumbed to the venom of *Heloderma*. The fact that little is known as to the pathogenesis of hemorrhagic erosions and the possibility of their development into typical gastric ulcer increases the importance of this work. The researches of Gay and Southard† on serum anaphylaxis, of Bolton‡ on gastrotoxic serum, and of Rosenau and Anderson§ on production of gastric ulcer and hemorrhage by the subcutaneous injection of diphtheria toxin into guinea-pigs, together with the exhaustive anatomical study by Beneke of hemorrhagic erosions occurring in the human stomach, represent the most important contributions relating to this problem.

At a recent meeting of the German Association of Pathologists in Kiel, Beneke¶ called attention to the frequency of hemorrhagic erosions and cited 400 cases which he had collected. He pointed out the possibility of their development into typical gastric ulcer. He says surgeons have frequently, within the last ten years, noticed the occurrence of hemorrhage in the stomach as evinced by the so-called "black vomiting" and even occasionally death. Anatomically, hemorrhagic erosions of Cruveilhier are found in such cases, and frequently associated with them are punctate ulcers. 27 per cent of Beneke's collected cases were post-operative, the great majority having been observed after intraperitoneal operations. He claims the danger in their formation is the greater the more the field of operation approaches the ductus choledochus and cœliac ganglion. The remainder of cases were found in a great variety of diseases. From a morphologic point of view, he classifies his cases as follows: (1) Typical hemorrhagic erosions. (2) Typical ecchymoses without ulcer. (3) Simple ulcer, origin doubtful. (4) Ulcer plus ecchymoses. (5) Parenchymatous ecchymoses and apparent anatomical changes of the stomach.

Beneke believes that the lesion is a primary digestion; that hemorrhage is secondary, and that the origin of but a small proportion is in ecchymoses. In order that digestion may take place, some special injury to the mucosa must occur, and this usually is brought about through a lack of oxygen. For in-

*A preliminary report of this work was published in the University of Pennsylvania Medical Bulletin. 1909.
†Journ. Med. Research, July 1908., vol. xix.
‡Proceed. Roy. Soc., 1905–1906, series B, 77, p. 426; series B, 79, 1909.
§Journ. Infect. Diseases, 1907. ¶Verhandl. d. deutsch. Path. Gesellsch., Kiel, 1908.

stance, in the new-born dying of asphyxia, he found many small necrotic areas in the stomach. He believes that a local ischemia is reflexly produced through some irritation of the central nervous system. This condition, combined with the collapse of the circulation and increased gastric secretion, results in hemorrhagic erosion or ulceration. Beneke's views in the main, however, are purely hypothetical. His experimental evidence, consisting in (1) ligation of the cœliac artery, and (2) injection of adrenalin into the muscularis of the stomach, is inconclusive; nor is his reasoning on the basis of anatomical observations quite convincing. Beneke, therefore, merely suggested the primary digestive nature of these phenomena without any experimental proof. Furthermore, his theory as to the formation of these lesions is probably, in part at least, incorrect, as will be demonstrated later.

Bolton* reports some very interesting results in his work on the action of gastro-toxic serum. This serum, which is produced by the injection of the mucous membrane of the stomach of the guinea-pig into the rabbit, is not absolutely specific in the true sense of the word. It produces necrosis and ulceration of the mucous membrane of the stomach when injected subcutaneously into the guinea-pig, but Bolton was unable to demonstrate any effect upon the gastric glands *in vitro*. The gastric cytotoxin formed in response to the injection of the gastric-cells seems to be a very complex body—increasing the hemolytic power which is normally present in the blood—containing many precipitins, most of which are absolutely specific, and containing no specific agglutinin for the gastric granules. By means of this serum it seems impossible to establish a chronic lesion, unless there is a perpetuation of the acutely produced lesion either by (1) a secondary bacterial infection, or (2) hyperacidity of the gastric juice. The actual necrosis and ulceration is produced by the action of the gastric juice on a cell which is functionally damaged. Hyperacidity increases the tendency toward ulceration.

Gay and Southard,† in their studies on anaphylaxis, also report anatomical changes in the gastric mucosa. In studying the causes of death in serum intoxication, they found hemorrhages in a great many organs. The most massive and most frequently observed occurred in the stomach-wall. Thus 32 out of 41 guinea-pigs dying within 24 hours of the second or toxic injection showed gastric hemorrhage, while 59 out of the total of 86 showed the same phenomenon. They do not consider the lesions as specific for serum intoxication in an anatomical sense, but claim that focal cytolyses of wide distribution occur and that this especially characterizes the action of the toxic phase. Certain diffuse fatty changes take place in the gastric epithelia where the local action of the gastric juice can come into play.

Rosenau and Anderson also describe an acute lesion of the gastric mucosa, which they are inclined to regard as more or less specific because it is confined to the pyloric end of the stomach. In their studies on diphtheria toxin they found that 66 per cent of the 1,879 animals injected with this toxin and dying

*Proceed. Roy. Soc., 1905–1906, series B, 77, p. 425; series B, 79, 1909.
†Journ. Med. Research, July 1908, vol. XIX.

acutely from the injection showed lesions of the stomach. Turck failed to produce gastric ulcer by injection of the diphtheria toxin into the stomach-wall; only minute hemorrhagic foci were found in the duodenum. He also failed to produce gastric ulcer by the introduction of the toxin into the mesenteric vessels. He obtained, however, in ten weeks, necrosis in the duodenum and near the pylorus. Rosenau and Anderson produce these changes by direct subcutaneous injection of the diphtheria toxin in a dose sufficient to cause the acute death of the animal. When the toxin is completely neutralized by antitoxin the stomach shows no lesions. Lesions may occur in infections with *Bacillus diphtheriæ* as well as through the action of the toxins. These pathologic changes—ulceration and hemorrhage—always occur at the pylorus and are found in 50 per cent of the animals dying within 24 hours and in 75 per cent of those dying between the third and fourth day after the injection. They enumerate the steps in the formation of the ulcer: (1) congestion, (2) hemorrhage, (3) digestion.

These observations represent more or less acute conditions in which toxic agents are at work. Indirectly they have, as will be shown, some bearing on our work.

METHOD OF PRODUCING ACUTE TOXIC ULCER OF THE STOMACH.

The beginning of our investigation was the observation that the subcutaneous injection of the venom of the Gila monster (*Heloderma suspectum*) into guinea-pigs frequently produced ulceration and hemorrhage of the stomach. The dose of venom necessary to produce this change depended on the toxicity of the venom, and as this constantly fluctuates, the dose must vary with the toxicity. In the beginning of our experiments we found that 1 c.c. of fresh venom, diluted in the proportion of 1 part of venom to 19 parts of normal salt solution, was sufficient to cause death in from 2 to 4 hours after the animal had reached a state of marked intoxication. Later on, however, as much as 1.5 to 3 c.c. of diluted fresh venom was occasionally required for the purpose. The dose of dried venom which exhibited approximately the same toxicity was from 12 to 15 mg., and a much greater dose was frequently given. The average weight of the animals employed in our experiments was from 350 to 400 grams. In larger animals the dose must be increased in proportion to the increase in weight.

It is very important that the animal should be in a state of marked intoxication and remain in such a condition for a period of at least an hour. Under such circumstances the animal is in a stuporous condition or has tremors, convulsions, or later actual paralysis. This condition of intoxication is the most important factor in the production of gastric ulcer; the dose of venom must be chosen accordingly and the injections repeated with sufficient frequency to insure the continuance of such an effect for a certain time. Following these rules, we were able to produce gastric ulceration in 35 out of 41 animals injected with venom alone—a little over 85 per cent. Of the 15 per cent in which no change or only a doubtful change was visible, several had received insufficient doses, and in the case of several others the toxicity of the venom had been

below normal. This ulceration was independent of the state of dying, inasmuch as animals killed at a much earlier period showed the same change, but the changes were usually more marked in direct proportion to the degree of intoxication manifested by the animal.

The Typical Ulcer.

The typical ulcer, usually accompanied by hemorrhage, was, on exposure of the peritoneal surface of the stomach, generally seen as a purplish-red circumscribed patch. If ulcers were unaccompanied by hemorrhage—and this was by no means rare—they appeared as rounded grayish points. On opening the stomach, hemorrhage was usually seen; after removing the blood the characteristic ulcer appeared, varying from 1 to 8 mm. in diameter. Occasionally, by confluence, they would involve as much as 0.5 square inch of the gastric mucosa. They were always found in the cardia and fundus, and were never observed in the pyloric fifth of the stomach. They are found more frequently at the greater curvature than at the lesser, but were by no means excluded from the latter. Of interest is their occurrence in discrete clusters—most frequently on the anterior cardiofundal region—or near the center of the stomach around the greater curvature. This arrangement might be explained on the ground that each territory involved was nourished by a certain blood-vessel and its branches. We endeavored, therefore, to ascertain, by holding the entire mucosa up to the light, what relation these ulcers held to the distribution of the vessels, and found a certain proportion of the clusters bearing some relation to the vessels.

The ulcers, when typical, are round, sharply circumscribed, with sloping edges resembling a peptic ulcer in the stomach of man, with or without a hemorrhagic base. Frequently the ulceration reaches down to the muscularis, and the ulcer then has a translucent appearance when viewed from the peritoneal surface and has usually a number of hemorrhagic erosions combined with it. These erosions occur so frequently and are seen so constantly, especially when an animal is killed soon after injection, before a definite ulcer has had a chance to develop, that in all probability the hemorrhagic erosion represents an early stage of these ulcers. Indeed, all transitions can be found, from hemorrhagic erosion to distinct, large, and well-defined ulcers. These ulcers bear a striking resemblance to the ulcers described by Bolton,* and, to judge from his description, the appearance of both kinds is identical. The fact that Bolton's lesions were acute also favors this view of the identity of both varieties; but we do not know whether the condition of marked intoxication, so important in the production of venom ulcers, was equally present in his animals, nor whether the anatomical position of the ulcers was the same in both cases. Both of these ulcers differed from those produced by Rosenau and Anderson in that the latter were confined to the pyloric region. Furthermore, as will be shown later, hemorrhage was not the primary factor in the ulcers produced by the injection of venom.

*Proc. Roy. Soc., 1905-1906, series B, 77, pp. 425, 428; series B, 79, 1909.

Microscopically a section through a well-formed ulcer shows a sharply cut crater resembling a peptic erosion. The walls of the mucosa dip in on either side, disclosing the muscularis or submucosa as the floor of the ulcer. In the ulcer proper we find granular débris consisting of digested mucosa, food particles, and occasional hemorrhages. Frequently, however, the walls, instead of being sharply cut, sloped gradually, giving the ulcer the appearance of an excavation. Another form is seen where three-fourths of the mucosa takes a more or less basic stain and sloughs off, leaving a few rows of intact cells below. Frequently a number of polynuclear leucocytes are seen in the base of the ulcer, together with a little fibrin; but connective-tissue proliferation does not take place, which is in accordance with the acute character of the lesion. Usually hemorrhagic areas are seen between the sloughed-off part of the mucosa and the intact mucosa below. This might suggest hemorrhage as the primary cause of the ulcer, but far more frequently areas of mucosa are seen which take a more or less basic stain. Under the high power various stages of nuclear degeneration are seen in these areas. The large majority of the nuclei are shrunken and do not take the stain. The protoplasm of the gland cells is granular and frequently has entirely disappeared in the upper layers, leaving nothing but poorly stained, shrunken nuclei and connective-tissue fibrils. This area contrasts strongly with the gastric tubules below, in which the cells are intact.

Interesting is the fact that in these superficial areas, when all other elements are necrotic, the acid cells stain well and seem able to resist digestion much better than the parietal cells. This was observed in a number of instances, and is in accord with Bolton's observations on the effect of the gastrotoxic sera on the gastric cells *in vitro*.

Every intermediate stage has been observed from slight change with no sloughing of the mucosa to actual ulcer, and frequently without hemorrhage. In this very early stage not even capillary hemorrhages can be seen. On the other hand, small submucous hemorrhages have been observed without any change in the overlying mucosa. In a study of a great number of sections, many of them serial, only one marked instance of submucous hemorrhage was found, and in this case no change was visible in the overlying mucosa. In several instances where gastric ulceration and hemorrhage were present, parenchymatous hemorrhages into the spleen were also observed.

The possibility of hemorrhage occurring in parts of the small or large intestines was thoroughly studied. The entire gastro-intestinal tracts of about 25 animals were carefully examined. No case of extra-gastric hemorrhage was observed, except in one case in which a little blood-stained fluid was found in the small intestine.

The vessels in the vicinity of the ulcer are often thrombosed; and frequently a peripheral rim of leucocytes, due to a gradual slowing of the circulation, was seen in these thrombi. That these thrombi are not the cause of the ulcer, but are secondary, will be shown later. They seal the vessels and thus prevent hemorrhage. Frequently the capillaries immediately surrounding the ulcer are congested.

Effect of Pilocarpine and Atropine on the Production of Ulcers.

On the basis of these anatomical facts, we extended our work in order to clear up the causation of these ulcers by experimental means. We studied the influence of gastric secretion, the effect of the neutralization of the latter by sodium-carbonate solution, the relation of these ulcers to the distribution of the vessels, and the effect of thrombosis. Inasmuch as pilocarpine is supposed to increase gastric secretion, and atropine to lessen it, it was of interest to observe in what way they could modify the ulceration produced by venom.

Added interest was attached to these experiments, as the possibility of the excretion of venom into the stomach as a cause of the ulceration had to be considered. We therefore injected three sets of animals, one with venom alone, one with atropine and venom, and one with pilocarpine and venom, and observed them under identical conditions. We found that those animals which received pilocarpine in addition to venom showed markedly increased ulceration and hemorrhage. In fact, the most marked cases of ulceration and hemorrhage seen in this work were observed after the combined use of pilocarpine and venom. For instance, in the case of guinea-pig 109 the stomach was completely covered with a great number of ulcers and hemorrhagic areas varying from 1 to 7 mm. in diameter. Atropine also increased the tendency toward ulceration, but this was not nearly as well marked as in the instance of pilocarpine. The addition of either substance, therefore, but especially of pilocarpine, to venom, increases the gastric ulceration. These results suggested the question as to what might be the effect of these alkaloids on the stomach without the addition of venom.

We therefore administered lethal doses of atropine and pilocarpine without venom. As much as 3.7 grains of atropine sulphate and 8 grains of pilocarpine hydrochloride were given subcutaneously in fractional doses with sufficient frequency to keep the animal in a markedly toxic state for several hours before death. In the two pilocarpine animals distinct ulceration and hemorrhage were present, while in two out of six atropine animals hemorrhagic erosion, and in one of these also a typical small ulcer, was found. Three of the six guinea-pigs that received atropine died so soon after the injection that the time was insufficient for an ulcer to form. In these experiments also the ulcers were formed if the animals had been in a markedly toxic state several hours before death.

Inasmuch as the ulceration and hemorrhage produced differed only quantitatively in these cases, we tried various other poisons, observing the precaution to inject the substance with sufficient frequency to keep the animal in a markedly toxic state for several hours before producing death. Paraldehyde, chloroform, 10 per cent phenol, and a saturated solution of magnesium chloride were all tried, as well as sodium fluoride and copper sulphate. Without going into detailed description, we may state that, with every poisonous substance, we were able to obtain, under proper conditions, some gastric lesion. This latter varied from distinct ulceration in the paraldehyde animals to the occasional hemorrhagic erosions seen in the chloroform or magnesium-sulphate animals.

At first it seemed doubtful whether any change was demonstrable after administration of the latter substance, but Doctor Loeb has since succeeded in producing lesions. It seems, therefore, certain that this reaction is in no wise specific for venom and that possibly all poisons, whether organic or inorganic, which will keep the animal in a state of marked intoxication will produce some change in the gastric mucosa. Whether this lesion is directly proportional to the depression caused by the poison we are not prepared to state. Certainly this is not invariably the case, inasmuch as magnesium-chloride animals, although markedly affected, showed very few lesions; usually, however, the severity of the lesion was in direct proportion to the degree of intoxication manifested by the animal.

Significance of Thrombosis in Gastric Ulceration.

As has been previously stated, thrombi were found in the vessels in the neighborhood of the ulcer, and as a decrease in the blood-supply had to be considered as a possible cause of the ulcers, and inasmuch as thrombi might be the cause of such a diminution in the blood-supply, we had to determine whether thrombosis was a primary or secondary factor in the formation of the ulcers. For this purpose we caused experimentally an incoagulability of the blood by injecting hirudin into the jugular vein, this being followed by the injection of venom. If ulcers occurred under such conditions, it was certain that thrombosis could be ruled out as an etiological factor. After several failures due to errors in technique, we succeeded in fulfilling all conditions in eight animals, and in all of them hirudin, instead of preventing ulceration and hemorrhage, actually augmented the latter. There were just as many ulcers present, and the hemorrhage, which was formerly in direct proportion to the degree of erosion of the vessels, assumed alarming proportions after the injection of this substance. Immediately on the death of the animal we were accustomed to withdraw 5 to 10 c.c. of blood from the heart, to test its coagulability and to control the action of the hirudin. In several instances the blood was not coagulated *in vitro* two hours after death. In one interesting case the animal had been in a very weak condition for some time before death. On opening its heart we were unable to withdraw more than a few drops of blood; the stomach, however, was distended with liquid blood and in the wall of the stomach were found several well-defined ulcers. The animal had practically bled to death into its stomach. In several animals a hemorrhagic mottling of the spleen was also observed.

From these observations we may conclude that thrombosis is not a primary factor in the production of the ulcers; that it is a secondary process due to changes in the circulation and to the liberation of tissue coagulins in the affected area; moreover, that it is beneficial, inasmuch as it prevents fatal hemorrhage.

IS THE ULCERATION DEPENDENT UPON PEPTIC DIGESTION?

Beneke believed that in the cases reported by him the ulcers were primarily caused by digestion, but no adequate experimental proof was offered for this interpretation. Bolton concluded that inasmuch as alkali prevented the for-

mation of an ulcer after the injection of gastrotoxic serum, the ulcers must be primarily digestive in character; and Rosenau and Anderson claim that the pyloric ulcers produced through the injection of diphtheria toxin were due to circulatory changes, congestion, and hemorrhage, followed by digestion.

Although according to our observation, macroscopically and microscopically, hemorrhage is nearly always a concomitant of the ulcers of the stomach, it is questionable whether the latter are primarily hemorrhagic. In order to decide this question we introduced into the stomach of a guinea-pig 14 c.c. of a 4 per cent sodium-bicarbonate solution through a stomach-tube, and injected the venom subcutaneously. This was the method used in the beginning. Later, we gave two injections of 10 c.c., one hour apart, in order to insure constant contact of the gastric mucosa with alkaline fluid. This mode of procedure fulfilled two indications: (1) it completely neutralized the gastric juice, and (2) it constantly moistened the gastric mucosa with alkaline fluid. We found that alkali markedly inhibited, and in the great majority of cases actually prevented, the formation of ulcers. Thus, in 30 animals in which alkali and venom were administered, 6 showed ulceration, 20 per cent; while over 80 per cent in the same number of animals in which venom alone and no alkali had been given showed ulceration. Not only was this the case, but in 4 of the 6 the ulceration was single, and in the other 2 animals the ulcers were very small, while in the controls the ulcers were large and multiple. Besides, in 2 of these 6 only 10 c.c. of alkaline fluid had been injected, and most of the fluid had probably passed over into the duodenum at the time the ulceration took place. The quantity of alkali was, therefore, insufficient. Traumatisms caused by the stomach-tube also could not be excluded. In the last series of 6 animals in which the technique was most perfect, the 3 animals with toxin and alkali showed absolutely no lesions, while the controls showed marked ulceration and hemorrhage.

These experiments indicate, therefore, that digestion is the primary factor in the formation of these ulcers. In favor of this interpretation we may likewise cite the observation that in a number of instances, as stated above, beginning necrosis or digestion could be observed microscopically without any preceding hemorrhage. Occasionally, however, hemorrhage occurred independently of ulceration; but an intact epithelial layer covering such an area makes it improbable that such a primary hemorrhage has much significance in the production of the ulcers. Our experiments do not permit us to make any definite statement as to the cause of the primary digestion, but we are inclined to believe that a malnutrition of certain areas of the gastric mucosa is brought about by a slowing of the circulation, especially as we have observed the ulceration in discrete areas and with especial frequency in those animals in which the phenomena of collapse were apparent. It is also possible that toxic substances may pass directly from the capillaries to the surrounding mucosa, change the vital processes of these cells, and lower their resistance to peptic digestion. Beneke's view, advocated first by Klebs, that spasm of the vessels with resulting ischæmia is responsible for tissue digestion, seems improbable,

inasmuch as it is questionable whether such a variety of poisons should all cause a constriction of the vessels, especially if we consider that several of these substances were vasodilators. That fatty degeneration of the glandular elements, such as described by Gay and Southard, is not the cause of the ulceration is evidenced by our inability to demonstrate any such change in sections through ulcerated areas stained by osmic acid.

It remains for further experiments to determine whether or not it is possible to establish a chronic lesion. In one instance an animal which had been injected with venom several months previously was killed and a somewhat older ulcer found, which under the microscope revealed a base infiltrated with round cells; no attempt at healing was observed. It is not unlikely that, if the animal remains alive, such an acute toxic ulcer may become chronic, especially if it be perpetuated by a bacterial infection, hyperchlorhydria, or anemia.

Total number of guinea-pigs, excluding those used for testing the strength of the venom, 127.
Of 41 injected with venom alone, 35 showed ulcer (85 per cent).
Of 30 animals injected with venom and alkali (or with venom + atropine and alkali), 6 showed ulcer (20 per cent).
Pilocarpine, 14 animals:
Control (venom alone), 4 animals, 4 showed ulcer (100 per cent).
Venom and pilocarpine, 8 animals, 8 showed ulcer (100 per cent).
Pilocarpine alone, 2 animals, 2 showed ulcer (100 per cent).
Atropine, 34 animals:
Control (venom alone), 9 animals, 8 showed ulcer.
Venom and atropine, 6 animals, 6 showed ulcer.
Venom, atropine, and alkali, 13 animals, 4 showed ulcer (1 of these cases was doubtful).
Atropine alone, 6 animals, 2 showed ulcer (2 died too early).
$MgCl_2$, 4 animals, 1 showed hemorrhagic erosion.
Paraldehyde, 2 animals, 2 showed ulcers.
Phenol, 2 animals, 2 showed ulcer.
Chloroform, 2 animals, 2 showed ulcer and hemorrhagic erosion.

In addition several animals were injected with sodium fluoride, $CuSo_4$, and $MgCl_2$ with positive results.

Hirudin experiments, 14 animals:
Hirudin + venom, 8 animals, 8 showed ulcer.
Controls (venom alone), 5 animals, 5 showed ulcer.

CONCLUSIONS.

After subcutaneous injections of the venom of *Heloderma* into guinea-pigs, gastric ulcer and hemorrhagic erosions are found in approximately 85 per cent of all the animals used. These ulcerations are aggravated by atropine as well as by pilocarpine; it is, therefore, unlikely that ulceration is due to the excretion of the venom through the intact mucosa. Various poisonous substances, otherwise differing widely in their chemical character and pharmacological actions, may all produce similar changes in the gastric mucosa. The effect of these substances is not a specific, but is probably an indirect one, acting either through a weakening influence on the circulation or through a direct injurious effect on the cells of the mucosa. The degree of efficacy of these substances is, on the whole, parallel to their general toxic effect or to the extent to which they affect the general vitality of the animals. There exist, however, marked differences in the tendency of these substances to produce ulceration (*cf.* especially magnesium chloride and paraldehyde).

In the large majority of cases both hemorrhage and ulceration are found in the affected areas. The fact that in a large majority of cases the introduction of alkali into the stomach is able to prevent the occurrence of ulceration and hemorrhage renders it almost certain that usually digestion is primary and that in many cases hemorrhage is merely the result of secondary erosion of the vessels. The results of microscopic observation favor this interpretation.

In the course of ulceration, neighboring blood-vessels become occluded by thrombi. This is secondary and not primary, inasmuch as we were able to show that experimentally produced incoagulability of the blood failed to prevent the occurrence of ulcers. Thrombi are, therefore, not the cause of ulceration, but this secondary thrombosis must be considered as a beneficial process, inasmuch as it prevents fatal hemorrhage.

These facts throw light on hemorrhagic erosions in man, which are, in all likelihood, due to primary digestion. They also indicate that such lesions may be transformed into typical gastric ulcer; at least such is the case in guinea-pigs subjected to the influence of different toxic substances. Whether chronic round ulcer can be produced by a perpetuation of such a condition remains to be proved.

VII.

HISTOLOGICAL CHANGES OF THE CENTRAL NERVOUS SYSTEM PRODUCED BY HELODERMA VENOM.

By Samuel Leopold.

HISTOLOGICAL CHANGES OF THE CENTRAL NERVOUS SYSTEM PRODUCED BY HELODERMA VENOM.

By Samuel Leopold.

The effect of the venom of various snakes upon the cerebro-spinal system has been studied histologically only since 1900. It will not be necessary to review the literature, as this has been done in Noguchi's work on Snake Venoms: An Investigation of Venomous Snakes with Special Reference to the Phenomena of their Venoms (Pub. No. 111, Carnegie Institution of Washington, 1909). It was of interest to compare the action of snake venom with the effect of heloderma venom upon the central nervous system.

Dr. Leo Loeb kindly placed at my disposal the brains and spinal cords of rabbits and guinea-pigs which had been injected with varying doses of the venom of *Heloderma*. The inoculations were either given subcutaneously or into the peritoneal cavity. The dose varied from 25 to 60 mg. Some animals were given but one injection, others two, and in one instance four celloidin capsules had been introduced intraperitoneally and the poison was allowed to act during a period of 28 days. The animals lived from 5 minutes to several weeks. This material for study was placed in 10 per cent formalin or in Muller's fluid, and the sections were stained usually with thionin (Nissl method) and with hemalum. In one animal (rabbit B II, living 13 days), sections of the spinal cord were stained by the Marchi method.

In most cases only the medulla and spinal cord were examined (guinea-pigs 16, 21, 22, 18, A^4; rabbit B^2). In four cases the cerebral cortex cerebellum, pons, medulla, and spinal cord were studied (rabbits Nos. 1, B I, and a^8). In one instance (guinea-pig a^1) sections were taken from every portion of the brain.

For control a healthy guinea-pig's brain was sectioned at various levels from the frontal lobes to the spinal cord, and stained by the Nissl method.

The earliest change noted in our animals occurred after 45 minutes (guinea-pig 18). Some of the cells in the anterior horn of the spinal cord took the stain a little more intensely and diffusely. The medulla and spinal cord of several animals dying at shorter intervals than 45 minutes failed to reveal any changes in their cells. Lamb and Hunter found with the venom of *Naja* definite changes only when the animals lived longer than 2 or 3 hours, but the venom of *Enhydrina* produced pronounced chromatolytic changes in 90 minutes, while Kilvington found chromatolysis with the venom of the tiger-snake if the animal survived the injection of the poison for over 3 hours.

In rabbit No. 1, which lived 75 minutes, some of the ganglion-cells of the spinal cord, medulla, and a few of the Purkinje cells showed an early disintegration of the Nissl bodies. A fine powder was seen around the nucleus, which

extended toward the periphery. Some of the cells showed a slight swelling. This latter feature was present in both acute and chronic cases, but seemed a more constant feature in the various sections of the chronic cases. This swelling of the cell does not seem to be a characteristic feature in the pathological findings of others. No mention of it is made by Lamb, Hunter, or Kilvington.

The disintegration of the Nissl bodies was the most constant finding in the acute cases, but it is not characteristic, being also found in the chronic cases.

In none of our acute cases was an eating-out of the cell seen or the complete dissolution of its cytoplasm, etc., as described by Lamb and Hunter. The process after injection of heloderma venom seemed much milder when compared with the effect of some of the snake venoms. In the ganglia-cells of our animals the nuclei were for the most part centrally placed and could always be recognized. They were misplaced only in exceptional cases.

Chromatolysis was a constant feature in the animals living from 48 hours to several days (guinea-pigs A_1, A_4; rabbits B^1 and B^2). In none of the cells was the outline lost; the nuclei were always visible and showed no change in form. These changes corresponded to those of Kilvington, Bailey, Lamb, and Hunter, save that in our cases the cells were in some instances swollen.

Another change which we found and which was not described by the above authors was the presence of minute areas of hemorrhage in two of our cases (rabbit a^3 and guinea-pig A^1). These hemorrhages were small and scattered through the entire structure of the medulla (rabbit a^3), while in guinea-pig A^1 they were noted around the central canal of the cord. These hemorrhages have not been sufficiently constant in our series to conclude that they are characteristic for the venom of heloderma. In guinea-pig A^1 the animal had convulsions prior to its death, and the convulsions may possibly be responsible for the hemorrhages.

We observed a round cell infiltration in two of the chronic cases (rabbits B^2 and A^8); in the former case (B^2) the cell infiltration was found around the posterior septum of the cord, and in the latter animal (a^8) around the blood-vessels. In the cases described by Lamb and Hunter, the cells were clustered around the disappearing ganglion-cells, the process being not unlike a neurophagia similar to that described by McKinas in poliomyelitis; while the infiltration in my cases was purely a chronic inflammatory process. As in the animals poisoned by snake venoms, we also found, after injection of heloderma venom, affections of the various cranial nuclei. In contradistinction to Lamb and Hunter's observations, we could not observe any special selective action of the venom on the third and fifth nuclei; in our entire series no preference could be detected for any one of the nuclei of the cranial nerves.

The changes in the ependymal cells were similar to those seen by previous authors in the case of snake venoms. A vacuolation of the cells was noted and the canal contained an excess of granular substance. In those cases in which hemorrhage occurred in the white substance (guinea-pig A^1 and rabbit a^3) a similar collection of red blood-corpuscles was observed in the canal.

The results obtained with the Marchi method were negative; no changes were found in the spinal cord of the animal living for 13 days. No changes in the myelin or in the axis cylinder were to be noted.

PROTOCOLS.

Guinea-pig No. 16.—25 mg. injected; dead in 8 minutes. Spinal cord and brain placed immediately in 10 per cent formalin. Sections embedded in celloidin. Stained with thionin (Nissl method). Examination of the medulla and spinal cord showed no changes in the ganglion-cells. The vessels were unaltered.

Guinea-pig No. 21.—20 mg. injected; dead in 15 minutes. Brain and spinal cord placed immediately in 10 per cent formalin. Sections embedded in celloidin. Stained with thionin. Examination of sections of the medulla and spinal cord taken at various levels showed no alteration in the cells or blood-vessels.

Guinea-pig No. 22.—20 mg. injected, dead in 30 minutes. Brain and spinal cord placed immediately in 10 per cent formalin. Portions of medulla and spinal cord were embedded in celloidin. Sections were cut at various levels and stained with thionin. Microscopical examination showed no changes in the cells or blood-vessels.

Guinea-pig No. 18.—25 mg. injected, dead in 45 minutes. Brain and spinal cord were placed immediately in 10 per cent formalin. Portions of the tissue were embedded in celloidin, cut, and stained with thionin. Some of the vessels in the medulla were dilated and congested. These vessels lie just beneath the fourth ventricle. Some of the cells of the spinal cord (anterior horn) and medulla (9th, 10th) took the stain a little more intensely.

Rabbit No. 1.—15 c.c. of venom injected; dead in 1 hour 15 minutes. Brain and spinal cord placed immediately in 10 per cent formalin. Portions of tissue were embedded in paraffin. The spinal cord, medulla, and cerebellum and pons were cut longitudinally. Sections were stained with thionin. Slight changes were noted in the cells of the spinal cord (anterior horn cells), the Purkinje cells in the cerebellum, and in a few cells in the nuclei of the cranial nerves. Owing to the manner of cutting the sections the identification of the cranial nuclei could not be assured, but it was evident that the toxin had no selective action, for cells were affected in the various groups of ganglion-cells throughout the medulla and pons. The changes to be noted were an early disintegration of the Nissl bodies into a fine powder, starting around the nucleus and proceeding to the periphery, a migration of some of the nuclei to the periphery, and a slight swelling of the cells. These changes were to be noted in only a few of the cells; the majority were normal.

Rabbit a^3.—25 mg. of venom injected; dead in 2 hours. Tissues hardened and embedded as in rabbit No. 1. The changes in the cerebellum, pons, and medulla were similar to those noted in rabbit No. 1, save that in the medulla numerous small hemorrhages were found.

Guinea-pig A_1.—January 7, 1909, 1 capsule with 0.5 c.c.; January 8, 1909, 5 p. m., dead. Died in 36 hours. Convulsions were noted, together with weakness and paralysis. Tissues and sections prepared as above. Portions were taken for study from several areas of the cerebrum, mid-brain, pons, cerebellum, medulla, and spinal cord. Many sections were cut from these areas. Some were stained with thionin and others with hemalum.

Spinal cord: Sections from the cervical region showed swelling of the cells in the anterior horn, a loss of staining reaction in the Nissl bodies, and displacement of the nuclei. Hemorrhages were to be noted above and below the central canal.

Sections from the thoracic region showed similar changes in the cells and marked congestion of the vessels throughout the white substance.

Medulla: Sections through upper and lower levels. The changes in the ganglion-cells were chromatolysis, swelling of the cells, and displacement of the nuclei. The ganglion-cells of the cranial nuclei seemed equally affected.

Pons: Similar changes were noted in the ganglion-cells of this region.

Mid-brain and basal ganglion: The cells in this region showed a similar picture, though not as many were affected.

Sections studied from various areas of the cerebral cortex showed little change.

Guinea-pig A_4.—24 mg. of venom in capsule. Dead in 48 hours. Sections of the medulla only studied. Quite a few of the ganglion-cells showed loss of staining power, indicative of a chromatolysis. The nuclei of the fifth, seventh, ninth, and tenth were affected alike.

Rabbit B_1.—2 capsules each 30 mg. placed in the peritoneal cavity. Sections fixed and stained as before.

Medulla: Chromatolysis of the Nissl bodies and displacement of the nuclei were seen in some of the ganglion-cells of the cranial nuclei. As these sections were cut longitudinally, the orientation of the various cranial nuclei could not be made out with certainty.

Cerebral cortex: Many of the Betz cells show breaking of the Nissl bodies, displacement of the nuclei, and shrinkage of the cell-body.

Rabbit B^2:—2 capsules, 30 mg. each, in the peritoneal cavity. Killed at the end of 13 days. Sections fixed in formalin and in Muller's fluid.

Spinal-cord sections stained with picric acid showed no changes.

Medulla: Sections stained by the Nissl method show the ganglion-cells of the tenth, ninth, seventh, and fifth cranial nuclei affected. Not all the cells were involved. There was loss of staining power in these cells, although their outlines were well defined. Shadow cells were seen with nuclei faintly present. A slight proliferation of connective tissue was noted in one area around the posterior septum in the medulla.

Rabbit a^3:—4 capsules (1 capsule 50 mg., 3 capsules 0.3 c.c.) given during a period of 28 days. Tissues placed in formalin 3 hours after death. In the medulla the cranial-nerve ganglion-cells showed some loss of staining power of the Nissl bodies. The fifth in this case seemed less involved than the tenth, ninth, and seventh. A few cells showed vacuolation and displacement of the nuclei. The vessels in the medulla were distended and congested. Slight round-cell accumulation was seen in the perivascular areas.

VIII.

HEMOLYTIC PROPERTIES OF HELODERMA VENOM.

By Elizabeth Cooke and Leo Loeb.

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HELODERMA VENOM HEMOLYSIS.

The venom of *Heloderma* bears in certain respects a resemblance to various snake venoms in which observers have shown more or less strong hemolytic properties. Van Denburgh and Wight* in a small series of experiments tested the hemolytic action of heloderma venom; they used the diluted fresh venom with washed dog-corpuscles and found that the venom, without any activating substance, was able to produce hemolysis. We have carried out a large series of experiments in which we studied the influence of the venom on hemolysis of various sorts of blood-corpuscles, of the combination of venom with various substances which are supposed to serve as activators—such as sera of various animals, lecithin, and sodium oleate—and the influence of various sera in inhibiting hemolysis.

HEMOLYTIC INFLUENCE OF HELODERMA VENOM.

Following the discovery of the complex character of snake-venom hemolysis by Flexner and Noguchi,† it was shown by Kyes‡ that cobra venom acts directly on certain blood-corpuscles, while it does not hemolyze certain others. Thus using twice-washed corpuscles, he found that the corpuscles of the ox, sheep, and goat were not dissolved by the venom, while those of the guinea-pig, dog, man, rabbit, and horse were dissolved. Similar results have been obtained with other snake venoms.

The venom of *Heloderma*, unlike the venom of *Cobra* or *Daboia*, and in common with certain other snake venoms, does not hemolyze corpuscles of any of the animals used in our experiments. We tested the corpuscles of the horse, ox, sheep, dog, rabbit, guinea-pig, turtle, frog, and *Heloderma*, using both fresh venom and dissolved dry venom. In the case of the fresh venom we mixed quantities varying between 0.01 c.c. and 0.25 c.c. with 1 c.c. of the corpuscles (which were always made into a 5 per cent suspension in either 0.85 per cent or 0.95 per cent sodium-chloride solution, depending upon the corpuscles used). When the dissolved dry venom was used we mixed quantities of the solution containing between 0.025 mg. and 0.2 mg. of the venom with the blood-corpuscles to be tested. The hemolytic test was kept for 2 hours in the thermostat and then left in the ice-chest over night. A reading of the various degrees of hemolysis was made on the following morning.

*Van Denburgh and Wight, Trans. Amer. Phil. Soc., 1898, xix, 199; Amer. Journ. Phys., iv, 1900–1901.
†Flexner and Noguchi, Jour. Exper. Med., 1902, vi, No. 3.
‡Kyes, Berl. klin. Woch., 1902, Nos. 38 and 39.

As stated above, no corpuscles were found to be susceptible to the venom. An example of one of the experiments with dog corpuscles and fresh venom is shown in the following protocol:

1 c.c. 5 per cent suspension of dog corpuscles.

Amount of heloderma venom:		Amount of heloderma venom:	
0.01 c.c.	0	0.06 c.c.	0
0.02 c.c.	0	0.1 c.c.	0
0.03 c.c.	0	0.15 c.c.	0
0.04 c.c.	0	0.2 c.c.	0
0.05 c.c.	0	0.25 c.c.	0

In all cases the blood-corpuscles had been washed four times in order to thoroughly free them of the blood serum.

It may be mentioned that in one case out of seven where guinea-pig corpuscles were mixed with a solution of the dry venom containing 0.2 mg. of venom, a trace of hemolysis appeared. It seems most probable that in this case we were dealing with some exceptional condition and that either the resistance of the corpuscles was less than normal or that the corpuscles had been injured in some manner.

INFLUENCE OF THE ADDITION OF LECITHIN TO HELODERMA VENOM ON THE HEMOLYSIS OF VARIOUS CORPUSCLES.

Kyes has shown that the addition of a solution of different commercial lecithins to cobra venom leads to the hemolysis of ox, sheep, and goat corpuscles, which are normally resistant to hemolysis by venom alone.

We have used three different specimens of lecithin, Agfa lecithin, Kahlbaum's lecithin aus eigelb, and a preparation of lecithin obtained through the kindness of Dr. Waldemar Koch, of Chicago. The lecithin was purified by dissolving it in ether, from which it was precipitated by the addition of a large quantity of acetone. This precipitated lecithin was then dissolved in methyl alcohol in the proportion of 1 gram of lecithin to 100 grams of methyl alcohol. From this stock solution of lecithin (1 per cent solution) a more dilute solution was made; 1 c.c. of the 1 per cent methyl-alcoholic lecithin solution was mixed with 99 c.c. of 0.85 per cent or 0.95 per cent sodium-chloride solution. The suspension contained 0.0001 gm. of lecithin in every cubic centimeter.

A few preliminary experiments were made to compare the three specimens of lecithin. In these experiments guinea-pig corpuscles were used.

Hemolysis of guinea-pig corpuscles by lecithin.

[1 c.c. of 5 per cent suspension of guinea-pig corpuscles.]

Amt. of lecithin.	Agfa.	Kahlbaum.	Chicago.	Amt. of lecithin.	Chicago.	Agfa (filtered).	Agfa (unfiltered).
mg.				*mg.*			
0.1	Total.	Total.	Total.	0.05	××	×	×
0.05	××	××	××	0.025	×	0	0
0.025	0	×	×				

We found the Chicago and Kahlbaum lecithin possessed distinctly stronger hemolytic properties than the Agfa lecithin. Furthermore, filtering the solution does not appear to diminish its hemolytic properties.

In another series of experiments we tested the activating properties of the various specimens of lecithin. A combination of the lecithin and venom was added to the washed guinea-pig corpuscles. The mixture was kept in the thermostat for 2 hours and left in the ice-chest over night.

Hemolytic action of heloderma venom and lecithin.

[1 c.c. of 5 per cent suspension of guinea-pig corpuscles, 0.1 mg. of heloderma venom.]

Amt. of lecithin.	Agfa.	Kahlbaum.	Chicago.	Amt. of lecithin.	Chicago.	Agfa (filtered).	Agfa (unfiltered).
mg.				*mg.*			
0.05	Total.	Total.	Total.	0.05	Total.	Total.	Total.
0.025	××	Total.	Total.	0.025	Total.	××	××
0.0125	0	×	×	0.0125	×	0	0
0.0065	0	×	0	0.00063	0	0	0

We found the combination of lecithin and venom produced hemolysis of the guinea-pig corpuscles. The venom alone caused no hemolysis, and the lecithin alone in quantities of either 0.025 mg. of the Agfa lecithin, or 0.0125 mg. of the Chicago or the Kahlbaum product were without effect, yet the combination of these substances produced a hemolysis which appears to be analogous to that produced by the cobra-venom-lecithin combination. It may be noted that in our experiments it was necessary to add to the venom a quantity of lecithin equal to half as much as the hemolytic dose of lecithin when not mixed with venom, in order to hemolyze the guinea-pig corpuscles, while Kyes states that $\frac{1}{200}$ of the hemolytic dose of lecithin when added to cobra venom was sufficient to activate the hemolysin. In many other cases, however, we found that one-quarter or even one-tenth of the hemolytic dose of lecithin was sufficient to hemolyze the blood corpuscles when combined with heloderma venom.

Having found that the combination of lecithin with venom produced hemolysis, we studied the effects of this combination on eight kinds of blood-corpuscles, namely, those of the ox, sheep, dog, rabbit, guinea-pig, turtle, frog, and heloderma. In the majority of these experiments Agfa lecithin was used as activator.

Ox Corpuscles.

In these experiments we used fresh venom as well as the dissolved dry venom.

[2 c.c. of 5 per cent suspension of ox corpuscles.]

Amt. of lecithin.	Lecithin.	Lecithin + 0.05 c.c. venom.	Amt. of lecithin.	Lecithin.	Lecithin + 0.1 mg. venom.
mg.			*mg.*		
0.4	Total.	Total.	0.1	×	Total.
0.2	×××	Total.	0.05	×	××
0.1	××	Total.	0.02	0	×
0.05	×	×××	0.01	0	0
0.025	0	××	0.004	0	0
0.0125	0	×	0.002	0	0
0.006	0	0	0.001	0	0

From these experiments it appears that the fresh venom hemolyzes the ox corpuscles with a trifle less lecithin than the dissolved dry venom. Further-

more, in the experiments with the fresh venom the addition of a quantity equal to only one-quarter the hemolytic dose of lecithin was needed in order to cause hemolysis, while with the dissolved dry venom a quantity equal to two-fifths of the hemolytic dose of lecithin was added.

We also tested the hemolytic doses of venom and lecithin with a constant quantity of lecithin and variable doses of venom, and found that when minute quantities of venom were added to a quantity of lecithin equal to two-fifths of the hemolytic dose, the ox corpuscles were hemolyzed.

2 c.c. of 5 per cent suspension of ox corpuscles.

[Lecithin, 0.02 mg. added to each tube.]

Fresh venom.	Dry dissolved venom.
0.4 c.c.....Total.	0.2 mg.....XXX
0.2 c.c.....Total.	0.1 mg.....XX
0.1 c.c.....XXX	0.05 mg.....XX
0.05 c.c.....XX	0.02 mg.....X
0.025 c.c XX	0.01 mg.....X
0.0125 c.c.....X	0.004 mg 0
0.006 c.c.....X	0.002 mg.....0

SHEEP CORPUSCLES.

A similar series of experiments was carried out with sheep corpuscles.

2 c.c. of 5 per cent suspension of sheep corpuscles.

Amt. of lecithin.	Lecithin.	Lecithin +0.05 c.c. venom.	Amt. of lecithin.	Lecithin.	Lecithin +0.1 mg. venom.	Amt. of lecithin.	Lecithin.	Lecithin +0.2 mg. venom.
mg.			*mg.*			*mg.*		
0.4	XXX	Total.	0.1	0	XX	0.5	XXX	Total.
0.2	XXX	Total.	0.05	0	X	0.2	XXX	Total.
0.1	XX	XXX	0.02	0	0	0.1	X	Total.
0.05	X	XXX	0.01	0	0	0.05	0	XXX
0.025	0	X	0.004	0	0	0.02	0	XX
0.0125	0	X	0.002	0	0	0.01	0	X
0.006	0	0	0.001	0	0			

From these experiments we may conclude that, to lecithin alone, the sheep corpuscles are more resistant than the ox corpuscles, and that the latter are more resistant than the guinea-pig corpuscles.

When venom and lecithin are mixed the sheep corpuscles are hemolyzed by quantities of lecithin smaller than the hemolytic dose of unmixed lecithin. Thus with fresh venom we obtained hemolysis of the corpuscles with a quantity of lecithin equal to one-quarter the usual hemolytic dose; with the dissolved dry venom (0.2 mg.) about one-tenth the usual hemolytic dose of lecithin was required.

2 c.c. of 5 per cent suspension of sheep corpuscles, lecithin 0.02 mg. added to each tube.

Fresh venom.	Dry venom.
0.4 c.c.....XX	0.2 mg.....0
0.2 c.c.....X	0.1 mg.....0
0.1 c.c.....0	0.05 mg.....0
0.05 c.c.....0	0.02 mg.....0
0.025 c.c.....0	0.01 mg.....0
0.0125 c.c.....0	0.004 mg.....0
0.006 c.c.....0	0.002 mg.....0

With a constant relatively small quantity of lecithin relatively very large doses of venom are required in order to cause hemolysis—much larger doses than were required for the hemolysis of ox and guinea-pig corpuscles; while with a constant dose of venom more lecithin is required for the hemolysis of sheep than of ox corpuscles. In a combination of lecithin and venom more of either of these two substances is required for the hemolysis of ox than of guinea-pig corpuscles.

Dog Corpuscles.

2 c.c. of 5 per cent suspension of dog corpuscles.

Amt. of lecithin.	Lecithin.	Lecithin+ 0.05 mg. venom.
mg.		
0.6	Total.	Total.
0.4	Total.	Total.
0.2	×××	Total.
0.1	×	Total.
0.05	0	×××
0.025	0	××
0.0125	0	0

In spite of the relatively small quantity of venom used, the addition of one-quarter of the hemolytic dose of lecithin to the venom is sufficient to cause hemolysis of dog corpuscles.

When the quantity of venom used was variable and only small quantities of lecithin were used, very small quantities (0.006 mg.) of venom were sufficient to cause partial hemolysis of the dog corpuscles, indeed somewhat less than was necessary to cause partial hemolysis of ox corpuscles (0.01 mg.), and very much smaller quantities than caused hemolysis with sheep corpuscles (0.2 c.c. fresh venom, none with 0.2 mg. dry venom).

2 c.c. of 5 per cent suspension of dog corpuscles.

Amt. of venom.	Venom+ 0.02 mg. lecithin.	Venom+ 0.01 mg. lecithin.	Amt. of venom.	Venom+ 0.02 mg. lecithin.	Venom+ 0.01 mg. lecithin.
0.6	Total.	××	0.025	××	×
0.4	×××	××	0.0125	×	×
0.2	×××	××	0.006	×	×
0.1	××	×	0.003	0	0
0.05	××	×	0.0015	0	0

Rabbit Corpuscles.

2 c.c. of 5 per cent suspension of rabbit corpuscles.

Amt. of lecithin.	Lecithin.	Lecithin +0.2 mg. venom.	Amt. of lecithin.	Lecithin.	Lecithin +0.2 mg. venom.	Amt. of lecithin.	Lecithin.	Lecithin +0.1 mg. venom.
mg.			*mg.*			*mg.*		
0.5	×××	Total.	0.02	0	×	0.2	0	0
0.2	×	×××	0.01	0	×	0.1	0	0
0.1	×	×××				0.05	0	0
0.05	0	××				0.02	0	0
						0.01	0	0

The reaction of the corpuscles to the venom and lecithin varied considerably. In certain cases, indeed, in most cases, the corpuscles were resistant to

the lecithin hemolysis and in such cases we were also unable to produce hemolysis with the venom-lecithin mixture. However, in one case in which the corpuscles were less resistant than usual, we found that the combination of venom and lecithin caused hemolysis of the corpuscles even if a quantity of lecithin equal to only one-tenth the dose hemolytic for rabbit corpuscles was added. The lecithin activates the venom for rabbit corpuscles as it does for ox, sheep, and dog corpuscles.

In testing the hemolytic action of a constant quantity of lecithin and a diminishing quantity of venom we were unable to overcome the great resistance of the rabbit corpuscles, and were thus unable to carry out satisfactory experiments.

Guinea-Pig Corpuscles.

In our experiments, the guinea-pig corpuscles were fairly resistant to hemolysis by lecithin alone, 0.1 mg. of lecithin being required for partial hemolysis. If, however, lecithin was added to 0.1 mg. of heloderma venom the corpuscles were hemolyzed after addition of only 0.02 mg. of lecithin, only one-fifth of the usual hemolytic dose.

2 c.c. of 5 per cent suspension of guinea-pig corpuscles.

Amt. of lecithin.	Lecithin.	Lecithin +0.1 mg. venom.
mg.		
0.4	×××	Total.
0.2	××	Total.
0.1	×	×××
0.04	0	××
0.02	0	×
0.008	0	0
0.004	0	0

With a constant quantity of lecithin and diminishing quantities of venom, very small quantities of venom were able to cause hemolysis; even as little as 0.01 mg. of venom, added to the same quantity of lecithin, was sufficient to cause hemolysis. It will be seen that in these experiments the degree of hemolysis depended largely upon the amount of lecithin, while, for instance, 0.4 mg. of venom plus 0.01 mg. of lecithin caused only moderate hemolysis, a similar quantity of venom added to 0.1 mg. of lecithin (which of itself causes a trace of hemolysis) caused complete hemolysis of the corpuscles.

2 c.c. of 5 per cent suspension of guinea-pig corpuscles.

Amt. of venom.	Venom+ lecithin 0.1 mg.	Venom+ lecithin 0.04 mg.	Venom+ lecithin 0.01 mg.
mg.			
0.04	Total.	×××	××
0.2	Total.	×××	××
0.1	×××	××	××
0.05	××	××	××
0.025	××	×	×
0.01	××	×	×
.....	×	0	0

Turtle Corpuscles.

Turtle corpuscles were distinctly resistant to the hemolytic action of lecithin; 20 mg. of lecithin were not sufficient to cause hemolysis of the turtle corpuscles.

2 c.c. of 5 per cent suspension of turtle corpuscles.

Amt. of lecithin.	Lecithin.	Lecithin+ fresh venom, 0.015 c.c.	Lecithin+ dry venom, 0.5 mg.	Amt. of lecithin.	Lecithin.	Lecithin+ fresh venom 0.015 c.c.	Lecithin+ dry venom, 0.5 mg.
mg.				*mg.*			
20	0	.....	.....	0.25	0	Total.	Total.
8	0	Total.	.....	0.125	0	Total.	Total.
6	0	Total.	.....	0.06	0	Total.	Total.
4	0	Total.	.....	0.03	0	Total.	.....
2	0	Total.	.....	0.015	0	×	.....
1	0	Total.	.....	0.0075	0	0	.....
0.5	0	Total.	Total.				

If, on the other hand, much venom (0.5 mg. of dry venom or 0.015 c.c. of fresh venom) was mixed with lecithin the turtle corpuscles were hemolyzed with very small amounts of lecithin. Thus the addition of 0.015 mg. of lecithin to the fresh venom was sufficient to cause slight hemolysis; while 0.06 mg. of lecithin, the smallest quantity of lecithin added to dry venom, caused complete hemolysis of the turtle corpuscles. Simultaneously with these experiments with fresh venom a similar series with guinea-pig corpuscles was carried out, our object being to compare the susceptibility of turtle and guinea-pig corpuscles to the mixture of lecithin and venom. While turtle corpuscles were distinctly more resistant to the action of lecithin alone, we found that the guinea-pig corpuscles and turtle corpuscles were hemolyzed by nearly the same amounts of venom and lecithin. (In this experiment turtle corpuscles were moderately hemolyzed by a mixture containing 0.01 c.c. of fresh venom and 0.06 mg. of lecithin, while guinea-pig corpuscles were practically completely hemolyzed by a similar mixture of these substances.)

Frog Corpuscles.

Only one experiment was carried out with frog corpuscles, and in this experiment we tested the influence of 0.1 mg. of venom to which lecithin was added and found that as little as 0.01 mg. of lecithin would activate this quantity of venom.

1 c.c. of 5 per cent suspension of frog corpuscles.

Amt. of lecithin.	Lecithin.	Lecithin+ 0.1 mg. of venom.
mg.		
0.05	0	×××
0.03	0	××
0.01	0	××
....	.	0

Heloderma Corpuscles.

Heloderma corpuscles, like turtle corpuscles, are resistant to hemolysis by lecithin; 45 mg. of lecithin are required to hemolyze 1 c.c. of heloderma cor-

puscles. In testing the influence of venom plus lecithin on the heloderma corpuscles we used corpuscles from normal animals as well as from those whose venom glands had been removed some time previously. At the same time a comparison was made with guinea-pig corpuscles.

Venom, 0.1 mg. in each tube.

Amt. of lecithin.	Guinea-pig corpuscles.	Heloderma corpuscles (1 c.c. normal).	Heloderma corpuscles (1 c.c. gland-less).
mg.			
30	Total very rapid.	Total.	Total.
20		Total.	Total.
10		Total.	Total.
5		Total	××
1		××	0

From these experiments it may be seen that the heloderma corpuscles are very much more resistant to hemolysis by venom and lecithin than are guinea-pig corpuscles, and (in one experiment) that the corpuscles of the glandless animal were somewhat more resistant than those of the normal heloderma.

In another experiment we found the heloderma corpuscles much more sensitive to the hemolytic action, by the addition of small quantities of lecithin, to both fresh and dissolved dry venom. We found the addition of $\frac{1}{400}$ of the usual hemolytic dose of lecithin to a small quantity of venom (0.001 c.c. or 0.1 mg.) was sufficient to cause hemolysis of 1 c.c. suspension of the heloderma corpuscles. The heloderma corpuscles are not resistant to heloderma venom-lecithin hemolysis.

1 c.c. of 5 per cent suspension of heloderma corpuscles (normal).

Amt. of venom.	Venom+ lecithin, 0.1 mg.	Venom+ lecithin, 0.2 mg.
0.003 c.c.	Total.	Total.
0.002 c.c.	Total.	Total.
0.001 c.c.	Total.	Total.
0.3 mg.	Total.	Total.
0.2 mg.	Total.	Total.
0.1 mg.	Total.	Total.

Summary.

Lecithin activates venom in the case of all corpuscles used in these experiments, namely, corpuscles of ox, sheep, dog, rabbit, guinea-pig, turtle, frog, and *Heloderma*. The susceptibility of the various corpuscles toward the venom-lecithin mixtures varies, but in general the corpuscles of the warm-blooded animals are hemolyzed if a quantity of lecithin equal to from one-half to one-tenth of the hemolytic dose of lecithin is added to the venom, while the corpuscles of the cold-blooded animals are hemolyzed by the addition to the venom of a quantity of lecithin equal to $\frac{1}{20}$ to $\frac{1}{400}$ of the hemolytic dose of lecithin. Since the quantity of lecithin which must be added to venom in order to cause hemolysis varies only within a relatively small range in the case of various cor-

puscles, whether of warm or cold blooded animals, the apparent difference in the activating power of venom in the case of warm and some cold-blooded animals is due to the greater resistance of the corpuscles of some of the cold blooded animals to the lecithin. With a constant quantity of venom an increase in the amount of lecithin causes an increase in hemolysis, while with a constant quantity of lecithin an increase in the amount of venom causes an increase in hemolysis.

In order to accomplish in both cases the same amount of increase in hemolytic effect it seems to be necessary to increase the amount of venom to a greater extent if the lecithin is kept constant than to increase the amount of lecithin if the venom is kept constant. The sensitiveness of various corpuscles of warm blooded animals toward the venom-lecithin mixture differs somewhat, guinea-pig and dog and ox corpuscles being somewhat more sensitive than rabbit and sheep corpuscles. An immunity of the heloderma corpuscles toward the hemolysis by their own venom does not exist, although the blood-corpuscles of *Heloderma* were found in a number of experiments to be somewhat more resistant than the corpuscles of other animals tested.

INFLUENCE OF THE ADDITION OF SODIUM OLEATE TO VENOM ON THE HEMOLYSIS OF VARIOUS CORPUSCLES.

Noguchi* and Von Dungern and Coca† have shown that sodium oleate combined with cobra venom acts in a manner similar to lecithin, in that it is able to activate the cobra venom. Since we have found that lecithin was able to activate the heloderma venom for the various kinds of corpuscles, it was of interest to determine whether sodium oleate would also act as an activator for this venom.

We used for this purpose a Merck & Co. preparation of sodium oleate in a 0.1 per cent solution in 0.85 per cent sodium chloride.

Ox Corpuscles.

We found that 1 c.c. of a 5 per cent suspension of ox corpuscles was easily hemolyzed by 0.002 gm. sodium oleate; 0.01 mg. of sodium oleate when added to 0.5 mg. of venom was sufficient to cause total hemolysis of the corpuscles. Addition of venom diminishes, therefore, 200 times the hemolytic dose of sodium oleate.

1 c.c. of 5 per cent suspension of ox corpuscles.

Quantity of sodium oleate.	Sodium oleate+ quantity of venom.	Result.	Quantity of sodium oleate.	Sodium oleate+ quantity of venom.	Result.
gm.	*mg.*		*gm.*	*mg.*	
0.01	....	Total.	0.00001	1.0	Total.
0.005	....	Total.	0.00001	0.5	Total.
0.002	....	Total.	0.00001	0.25	XXX
0.001	....	XXX	0.00001	0.1	X

*Noguchi. Jour. Exper. Med., 1907, IX, 436. †V. Dungern and Coca. Berl. klin. Woch., 1908, p. 348.

Sheep Corpuscles.

Sheep corpuscles which react to sodium oleate in the same manner as ox corpuscles, being dissolved by 0.002 gm. of sodium oleate, were not hemolyzed by the mixtures of venom and sodium oleate used in our experiments. We have tested 0.01 mg. of sodium oleate plus 1 mg. of venom, but found no hemolysis. It is apparent that sheep corpuscles are more resistant to the action of venom plus sodium oleate than are the ox corpuscles.

Dog Corpuscles.

Dog corpuscles are hemolyzed by 0.4 mg. of sodium oleate but not by 0.2 mg. of this substance. The smaller quantity, namely, 0.2 mg., of sodium oleate is able to produce hemolysis after the addition of venom.

1 c.c. of 5 per cent suspension of dog corpuscles.

Amt. of sodium oleate.	Sodium oleate.	Sodium oleate + venom 0.05 mg.	Amt. of venom.	Venom + sodium oleate. 0.2 mg.
mg.			*mg.*	
1	Total.	....	0.4	××
0.7	Total.	....	0.2	×
0.5	Total.	....	0.1	0
0.4	Total	....	0.05	0
0.2	0	××		
0.1	0	0		
0.05	0	0		

With dog corpuscles sodium oleate has only a slight activating power for the venom, as it is necessary to add half or more than half the hemolytic dose of sodium oleate to the venom in order to produce hemolysis.

Rabbit Corpuscles.

Rabbit corpuscles are hemolyzed by 0.5 mg. of sodium oleate and the quantity of this substance which must be added to the heloderma venom in order to produce hemolysis is more than half of this quantity. Again the activating property of the sodium oleate is very slight.

Guinea-Pig Corpuscles.

Guinea-pig corpuscles are slightly hemolyzed by 0.125 mg. of sodium oleate. In the mixture of venom and sodium oleate about half as much sodium oleate is necessary to hemolyze the corpuscles.

1 c.c. of 5 per cent suspension of guinea-pig corpuscles.

Amt. of sodium oleate.	Sodium oleate.	Sodium oleate + venom. 0.125 mg.
mg.		
1	Total.	Total.
0.5	Total.	Total.
0.25	×××	Total.
0.125	×	×××
0.06	0	×××
0.03	0	0

Amt. of venom.	Venom + sodium oleate, 0.125 mg.
mg.	
2	Total.
1	Total.
0.5	Total.
0.25	××
0.125	××
0.06	××
0.03	×

Control, 0.125 mg.; sodium oleate, ×.

The hemolysis caused by 0.125 mg. of sodium oleate is less than the hemolysis caused by a similar quantity of sodium oleate plus venom.

Turtle Corpuscles.

The turtle corpuscles react to sodium oleate in a manner similar to the guinea-pig corpuscles; with 0.1 mg. of sodium oleate there is slightly less hemolysis of the turtle corpuscles than of the guinea-pig corpuscles; as was shown in parallel series carried out with guinea-pig and turtle corpuscles with similar quantities of sodium oleate or mixtures of sodium oleate and venom.

1 c.c. of 5 per cent suspension of turtle corpuscles.

Amt. of sodium oleate.	Sodium oleate.	Sodium oleate + venom, 0.02 c.c. (fresh venom).	Amt. of sodium oleate.	Sodium oleate.	Sodium oleate + venom, 0.02 c.c. (fresh venom).
mg.			*mg.*		
0.4	Total.	Total.	0.06	0	×
0.3	Total.	Total.	0.05	0	0
0.2	Total.	Total.	0.04	0	0
0.1	×	×××	0.03	0	0
0.09	0	×××	0.02	0	0
0.08	0	××	0.01	0	0
0.07	0	×			

After addition of venom the quantity of sodium oleate necessary to cause hemolysis was a little more than half the usual hemolytic dose, whereas a somewhat smaller dose of sodium oleate when added to the venom suffices to hemolyze the guinea-pig corpuscles.

Summary.

Sodium oleate serves as an activator for the heloderma venom, but the difference between the hemolytic dose of the sodium oleate alone and the activating dose of sodium oleate is very small. Addition of venom to sodium oleate diminishes the hemolytic dose of the sodium oleate approximately one-half in the case of the majority of corpuscles. There exists, therefore, an activating power of venom when combined with sodium oleate, but it is considerably smaller than in the case of lecithin.

ACTIVATION OF HELODERMA VENOM BY VARIOUS SERA.

Flexner and Noguchi,* who discovered that addition of serum to snake venom made the latter hemolytic, were inclined to identify the ordinary serum complement with the activating substance. Later the investigations of Kyes† and Calmette‡ suggested lecithin as the source of the venom-activating substance. The subsequent studies of Kyes and Sachs,§ however, made it very probable that besides the lecithin some other activating substance was present in various sera.

We have carried out a large series of experiments in which we tested the power of the various sera to serve as activators of the heloderma venom, and

*Flexner and Noguchi. Jour. Exper. Med. 1902, VI, No. 3.
‡Kyes. Berl. klin. Woch., 1902, Nos. 38 and 39.
†Calmette. C. R. Acad. Sci., 1902, 134, 1446.
§Kyes and Sachs. Berl. klin. Woch., 1903, XL, Nos. 1, 2, and 3.

in most cases we tested the action of the combination of the various sera with venom on the corpuscles of different animals.

We have found that the serum of the guinea-pig, rabbit, sheep, ox, *Heloderma*, or frog will not serve as activators for the heloderma venom.

The guinea-pig serum has been tested with washed sheep, dog, rabbit, and guinea-pig corpuscles, but failed to hemolyze any of these corpuscles with or without venom. Guinea-pig serum if added to ox corpuscles, either with or without venom, caused a minute trace of hemolysis in both series of tubes; no distinct difference could be detected between the hemolysis in the tubes containing serum alone and the combination of serum and venom; guinea-pig serum did not therefore serve as an activator for the venom.

Rabbit serum also was tested with rabbit, guinea-pig, ox, sheep, and dog corpuscles. With rabbit and guinea-pig corpuscles, no, or only a trace of, hemolysis was observed either with rabbit serum alone or with rabbit serum and venom. With ox and sheep corpuscles the rabbit serum alone, as well as the combination of serum and venom, produced slight hemolysis, which was, however, as marked in the control tubes as in the venom-serum tubes. In one case rabbit serum combined with the venom produced more hemolysis of dog corpuscles than the same quantities of the serum alone. In other cases, however, the rabbit serum, when combined with venom, did not hemolyze the dog corpuscles, so that in this isolated case, when rabbit serum seemed to serve as an activator for the venom, some accidental circumstances must have been active.

Sheep serum was tested with guinea-pig, rabbit, dog, sheep, and ox corpuscles. The ox corpuscles were slightly hemolyzed by sheep serum alone, as well as in combination with venom, but no difference was found between the degree of hemolysis in the two series of tubes. With the other corpuscles neither the sheep serum alone nor the mixture of sheep serum and venom produced hemolysis.

Ox serum was tested with the same corpuscles, namely, guinea-pig, rabbit, dog, sheep, and ox; it showed more marked hemolytic power than other serums, but did not activate the venom.

Frog serum was tested only with frog corpuscles, but did not cause hemolysis in combination with venom.

Heloderma serum was also tried with guinea-pig or heloderma corpuscles, and neither alone nor mixed with venom produced hemolysis. When heloderma serum was added to rabbit corpuscles, either alone or in combination with venom, the same degree of hemolysis occurred in both sets of tubes.

We may therefore conclude that the sera of guinea-pig, rabbit, ox, sheep, frog, or heloderma do not serve as activators for the heloderma venom. Calmette found in the case of cobra venom that unheated sera could not activate venom, but when sera was heated to a temperature above 62° C. it would activate venom.

We therefore tested 0.1 and 0.2 c.c. guinea-pig and rabbit serum heated to 63° C. for 30 minutes in combination with 0.1 and 0.2 mg. venom and 2 c.c. of a 5 per cent suspension of rabbit and guinea-pig corpuscles. In no case did

the heated serum show any activating power. We may therefore conclude that serum heated above 62° C. does not become an activator in the case of heloderma venom.

Dog serum was found to activate the heloderma venom. With ox and sheep corpuscles the activating property of the dog serum is slight, since the quantity of serum when combined with venom necessary to completely hemolyze either ox or sheep corpuscles will by itself cause slight hemolysis of both kinds of corpuscles.

2 c.c. of 5 per cent suspension of ox corpuscles.

Amt. of venom.	Venom + 0.1 c.c. dog serum.
mg.	
0.2	Total.
0.1	Total.
0.05	Total.
0.00	×

2 c.c. of 5 per cent suspension of sheep corpuscles.

Amt. of venom.	Venom + 0.1 c.c. dog serum.
mg.	
0.2	Total.
0.1	Total.
0.05	Total.
....	××

In the case of dog corpuscles the results are more striking, since a quantity of dog serum equal to half the hemolytic dose for dog corpuscles was sufficient to activate the heloderma venom.

2 c.c. of 5 per cent suspension of dog corpuscles.

Amt. of venom.	Venom + 0.1 c.c. dog serum.	Venom + 0.2 c.c. dog serum.
mg.		
0.5	Total.	Total.
0.25	Total.	Total.
0.125	Total.	Total.
0.06	Total.	Total.
0.02	××	Total.
.....	0	Slight.

2 c.c. of 5 per cent suspension of rabbit corpuscles.

Amt. of venom.	Venom + 0.1 c.c. of dog serum.
mg.	
0.5	Total.
0.2	Total.
0.1	Totul
0.05	0
....	0

1 c.c. of 5 per cent suspension of heloderma corpuscles.

Amt. of venom.	Venom + 0.2 c.c. dog serum.
mg.	
0.3	Total.
0.2	Total.
0.1	Total.
...	0

Rabbit corpuscles appeared to be slightly more resistant than the dog corpuscles to the hemolytic effect of venom and dog serum combined. The rabbit corpuscles, like dog corpuscles, were hemolyzed by 0.2 c.c. of dog serum. When the dog serum and venom were combined 0.1 c.c. of the former was sufficient when added to 0.1 mg. of venom to cause hemolysis. If only 0.05 mg. of venom was added to the dog serum no hemolysis took place.

Guinea-pig corpuscles were completely hemolyzed by 0.2 c.c. of dog serum alone. When venom and dog serum were combined 0.1 c.c. of the latter and 0.02 mg. of venom were sufficient to cause moderate hemolysis, while 0.1 c.c. of serum and 0.06 mg. of venom caused complete hemolysis of guinea-pig corpuscles.

Heloderma corpuscles were also hemolyzed by venom and dog serum. While 0.2 c.c. of dog serum did not hemolyze these corpuscles, this amount of serum was able when combined with the venom to cause hemolysis of the heloderma corpuscles. Smaller quantities of dog serum did not activate the venom for these corpuscles.

The hemolytic action of dog serum and venom upon turtle corpuscles is quite variable. In cases in which large quantities of dog serum were added to venom, the turtle corpuscles were partially hemolyzed, but the same quantities of dog serum alone also caused hemolysis, although slightly less marked.

1 c.c. of 5 per cent suspension of turtle corpuscles.

First experiment.		
Amt. of dog serum.	Dog serum.	Dog serum +0.1 mg. venom.
c.c.		
0.4	×	××
0.2	×	××
0.1	×	×
0.05	×	×

Second experiment.	
Amt. of venom.	Venom + 0.1 c.c. dog serum.
mg.	
0.4	0
0.2	0
0.1	0
0.05	0

In another experiment with corpuscles from another turtle in which dog serum was combined with diminishing quantities of venom, no hemolysis took place.

Horse Serum.

Horse serum as well as dog serum serves as an activator for venom. The action of venom and horse serum was tested upon guinea-pig, rabbit, and dog corpuscles, with similar results in each case. Horse serum alone has distinctly less hemolytic activity than dog serum, but it serves equally as well as an activator for the heloderma venom.

2 c.c. of 5 per cent suspension of guinea-pig corpuscles.

Amt. of horse serum.	Horse serum+0.1 mg. of venom.	Horse serum.
c.c.		
0.5	Total.	0
0.3	×××	0
0.1	×××	0
0.05	×	0

Turtle Serum.

Turtle serum, like horse and dog serum, activates the heloderma venom. This was tested on rabbit and guinea-pig corpuscles. When venom was combined with diminishing quantities of turtle serum these corpuscles showed

1 c.c. of 5 per cent suspension of rabbit corpuscles.

Amt. of turtle serum.	Turtle serum+0.1 mg. venom.	Turtle serum.
c.c.		
0.4	Total.	0
0.2	×××	0
0.1	××	0
0.05	××	0

1 c.c. of 5 per cent suspension of turtle corpuscles.

Amt. of turtle serum.	Turtle serum.	Turtle serum+0.1 mg. venom.
c.c.		
0.4	0	0
0.2	0	0
0.1	0	0
0.05	0	0

marked lysis. When the activating influence of turtle serum was tested with turtle corpuscles no hemolysis occurred. Turtle corpuscles seem to be very resistant to the combination of venom and turtle (or dog) serum.

COMBINATION OF HEATED SERA AND HELODERMA VENOM.

In testing the influence of heat on the activating properties of the various sera, especially guinea-pig, rabbit, sheep, or heloderma serum, it was found that 60° C. for 30 minutes did not transform them into activators.

Dog serum, when heated to 56°, 60°, or 70° C. for 30 minutes, lost its inherent hemolysins, but did not lose its activating property when combined with venom. It then hemolyzed guinea-pig, horse, dog, and heloderma; the last named corpuscles were, however, only slightly hemolyzed by this combination. As in experiments with unheated dog serum the addition of heated dog serum to venom did not hemolyze frog or turtle corpuscles.

Horse serum, when heated to 60° C. retained its action as an activator, and combined with venom caused hemolysis of horse, dog, rabbit, or guinea-pig corpuscles. The heated horse serum alone did not cause hemolysis of any of these corpuscles.

Turtle serum, when heated, was able to activate venom for guinea-pig and rabbit corpuscles, but not for turtle corpuscles. The quantities of these three sera that had to be added to venom to cause hemolysis were approximately the same before and after heating; the heating did not change the activating properties of these three sera to any marked extent.

Calmette has shown that in conjunction with cobra venom many sera heated to 62° C. were better able to cause hemolysis of red cells than the unheated sera. He believed that the heating destroyed a natural antihemolysin which was thermolabile, whereas the activating constituent was thermostable. This activating constituent he considered a "substance sensibilatrice."

Kyes found that heating changed the properties of the activating sera. Ox serum heated to 56° C. loses its activating power, but when heated to 65° C. or higher it regains its hemolytic power and is then more powerful than with the fresh serum. He also found that horse serum serves as an activator when fresh as well as when heated to 56° or 100° C., and he believed the activating substance of horse serum was lecithin. Our results make it certain that the ordinary serum complement is not concerned in the venom hemolysis, but that a certain thermostable substance already present in the fresh sera is responsible for the activating action of the sera.

INHIBITORY ACTION OF VARIOUS SERA ON HEMOLYSIS BY HELODERMA VENOM AND DIFFERENT ACTIVATORS.

We tested the inhibitory action of different sera (horse, guinea-pig, turtle, heloderma, and rabbit serum), heated and unheated, upon the hemolysis of erythrocytes of different species by the combination of venom and different activating substances.

We used horse, guinea-pig, turtle, heloderma, and rabbit serum and the corpuscles of guinea-pig, horse, *Heloderma*, turtle, rabbit, and dog. In the majority of experiments lecithin was the activator used.

Guinea-pig serum has approximately the same action whether heated or not heated. It inhibits hemolysis of guinea-pig, horse, heloderma, and turtle

corpuscles, but has no inhibitory influence on rabbit and dog corpuscles. In the case of dog corpuscles, the lysis of the corpuscles appears more slowly in the tubes to which heated guinea-pig serum is added than in those with fresh serum.

When 0.1 mg. of venom mixed with 0.05 mg. of lecithin was added to 2 c.c. of a 5 per cent suspension of guinea-pig, horse, or 1 c.c. of a 5 per cent suspension of turtle or heloderma corpuscles, hemolysis was complete or nearly complete, but when sufficiently large quantities of guinea-pig serum were added to these combinations hemolysis was prevented. When, for instance, 0.1 c.c. of guinea-pig serum, either heated or unheated, was added to mixtures containing either horse or guinea-pig corpuscles, no hemolysis occurred. The addition of 0.4 c.c. of serum was sufficient to completely prevent hemolysis with turtle or heloderma corpuscles, while 0.1 c.c. had some inhibitory effect.

Mixtures of 0.1 mg. of venom and 0.1 mg. of lecithin.

Amt. of guinea-pig serum.	2 c.c. 5 per cent suspension guinea-pig corpuscles.	2 c.c. 5 per cent suspension horse corpuscles.	1 c.c. 5 per cent suspension turtle corpuscles.	1 c.c. 5 per cent suspension heloderma corpuscles.
c.c.				
1.0	..	..	0	..
0.6	..	..	0	..
0.5	0	0	..	..
0.4	..	..	0	0
0.3	0	0	..	..
0.2	0	0	×××	..
0.1	×	0	×××	×
0.05	×	×	..	..
0.02	×	×××	..	..
....	Total.	Total.	Total.	Total.

With dog corpuscles no inhibitory action whatsoever could be observed when either heated or unheated guinea-pig serum was added. The same was true in the case of the rabbit corpuscles.

No difference existed between the action of heated or unheated guinea-pig serum in those cases in which an inhibitory action was demonstrated. Qualitatively and quantitatively their action was the same.

When heated horse serum was used as an activator instead of lecithin, guinea-pig serum (both heated and unheated) inhibited hemolysis, very markedly with horse corpuscles and slightly less markedly with guinea-pig corpuscles.

Mixtures of 0.1 mg. of venom and 0.1 c.c. of heated horse serum.

Amt. of guinea-pig serum.	2 c.c. 5 per cent suspension of horse corpuscles.	2 c.c. 5 per cent suspension guinea-pig corpuscles.
c.c.		
0.5	0	0
0.3	0	×
0.1	×	×
0.05	××	××
0.02	×××	×××
....	Total.	Total.

Mixtures of 0.1 mg. of venom and 0.1 c.c. heated dog serum and 2 c.c. of a 5 per cent suspension of guinea-pig corpuscles.

Amt. of guinea pig serum.	Result.
c.c.	
0.7	×
0.4	×
0.1	Total.
0.05	Total.
0.02	Total.
....	Total.

When dog serum heated to 56° C. was used as an activator, the inhibitory action was not so marked as when lecithin or horse serum was used as an activator, and we could only partially prevent hemolysis of guinea-pig corpuscles. In these experiments only heated guinea-pig serum was used.

With sodium oleate as an activator, the guinea-pig serum also inhibited the hemolytic action. In this case the inhibiting action of guinea-pig serum was not so strong as with lecithin, more pronounced than with dog serum and about equally strong with horse serum as an activator.

Summary.

Guinea-pig serum inhibits the hemolysis by lecithin and venom of guinea-pig and horse corpuscles, and only a little less markedly that of turtle and heloderma corpuscles. Hemolysis of horse corpuscles by venom and heated horse serum is also markedly inhibited, while hemolysis of guinea-pig corpuscles by similar mixtures is slightly less markedly inhibited. The action of venom and sodium oleate on guinea-pig corpuscles is considerably interfered with by guinea-pig serum but the action of heated dog serum and venom is only slightly affected. On the other hand, guinea-pig serum does not inhibit the hemolysis of dog corpuscles by venom and lecithin, nor can any influence of guinea-pig serum be observed when rabbit corpuscles were mixed with venom plus lecithin or horse serum. Unheated guinea-pig serum acts in the same manner as heated guinea-pig serum.

Heloderma serum, like guinea-pig serum, has marked inhibitory power, the unheated and heated serum acting in a similar manner, but the inhibitory power of the heated serum is, perhaps, slightly stronger than that of the unheated serum, as shown in the following :

Mixture of 0.2 mg. of venom plus 0.1 mg. of lecithin and 1 c.c. of a 5 per cent suspension of heloderma corpuscles.

Amt. of heloderma serum.	Heated serum.	Unheated serum.
c.c.		
0.5	0	0
0.3	0	0
0.2	0	0
0.1	0	0
0.05	0	×
....	Total.	Total.

Mixture of 0.2 mg. of venom and 0.1 mg. of lecithin.

Amt. of heloderma (heated) serum.	1 c.c. of a 5 per cent suspension turtle corpuscles.	2 c.c. of a 5 per cent suspension guinea-pig corpuscles.
c.c.		
0.6	0	..
0.5	..	0
0.3	0	0
0.1	××	×
0.05	Total.	××
....	Total.	Total.

It will be noted that heloderma serum exhibits a greater inhibitory power than guinea-pig serum, since 0.05 c.c. of the former is able entirely to prevent hemolysis, while 0.1 c.c. of guinea-pig serum allows a trace of hemolysis to take place, although more venom had been added in the experiment with heloderma venom.

The action of a mixture of venom and lecithin on turtle and guinea-pig corpuscles was also inhibited by the heated heloderma serum.

The influence of guinea-pig and heloderma serum are approximately the same in experiments in which guinea-pig corpuscles were mixed with lecithin and venom.

When heated dog serum was utilized as activator in place of the lecithin, the addition of heated heloderma serum in large quantities prevented hemolysis.

Mixture of 0.1 mg. of venom and 0.1 c.c. of heated dog serum and guinea-pig corpuscles (2 c.c. of a 5 per cent suspension).

Amt. of heloderma serum.	Result.
c.c.	
0.5	0
0.3	×
0.1	××
0.05	×××
....	Total.

Here again the inhibitory action of heloderma serum is more marked than that of guinea-pig serum, since 0.7 c.c. of the latter was not sufficient to completely prevent hemolysis, whereas 0.5 c.c. of the heloderma serum does prevent hemolysis by venom and dog serum.

From these experiments we may conclude that heloderma serum possesses a somewhat greater inhibitory power than guinea-pig serum and that it protects its own corpuscles best of all. It also seems that guinea-pig corpuscles are more strongly protected than turtle corpuscles by heloderma as well as by guinea-pig serum.

Horse serum, when combined with venom and lecithin, does not prevent hemolysis. The influence of these combinations was tested on horse, rabbit, dog, and guinea-pig corpuscles and both heated and unheated horse serum was used. The results were the same in all cases; no inhibition was evident, and indeed in most experiments hemolysis appeared more rapidly in the tubes containing serum, lecithin, and venom than in those containing only lecithin and venom. It is evident that horse serum does not inhibit hemolysis by venom and lecithin.

Turtle serum appears to have a slight inhibitory action when venom and lecithin act on turtle corpuscles. This holds good only in the case of the unheated serum.

Mixture of 0.1 mg. of venom and 0.1 mg. of lecithin.

Amt. of turtle serum.	1 c.c. of 5 per cent suspension turtle corpuscles and heated serum.	1 c.c. of 5 per cent suspension turtle corpuscles and unheated serum.	2 c.c. of a 5 per cent suspension guinea-pig corpuscles and heated serum.
c.c.			
0.5	×××	×	××
0.3	×××	×	××
0.1	Total.	××	×××
0.05	Total.	×××	×××
....	×××	×××	××

We can state that heated turtle serum does not inhibit hemolysis of turtle or guinea-pig corpuscles by venom and lecithin; indeed the addition of heated turtle serum seems to increase the hemolysis. It does appear, however, that unheated turtle serum inhibits, at least to some extent, the hemolysis of turtle corpuscles by lecithin and venom. The most plausible explanation for the action of turtle serum seems to be as follows: In turtle serum both an activating as well as an inhibiting substance exists. In heated turtle serum the activating substance prevails to a greater extent than in the unheated serum. If a combination of venom and lecithin is used the activating substance of turtle serum can act only to a slight extent, but it is able to neutralize the inhibiting substance.

Rabbit serum acts in a somewhat irregular manner, its action varying with different sera and different corpuscles.

With rabbit corpuscles the rabbit serum prevented hemolysis in almost every case; with guinea-pig corpuscles the rabbit serum prevented hemolysis in three out of five experiments; while with horse corpuscles hemolysis was prevented in only two out of five experiments.

Mixture of 0.05 mg. lecithin, 0.01 c.c. of venom, and 2 c.c. of suspension of corpuscles.

Amt. of heated rabbit serum.	Guinea-pig corpuscles.	Horse corpuscles.	Rabbit corpuscles.
c.c.			
0.6	0	×	0
0.4	×	×	0
0.2	×	××	0
0.1	×	Total.	0
0.05	××	Total.	×
....	Total.	Total.	Total.

The inhibitory action was quantitatively most marked when rabbit corpuscles were used, and least marked when horse corpuscles were used. As a rule when a certain specimen of rabbit serum did not protect the horse corpuscles it likewise had no, or only a very slight, protective action with the guinea-pig corpuscles.

Heated and unheated rabbit serum acted in the same manner; a certain serum which when heated inhibited the hemolysis of rabbit corpuscles by venom and lecithin acted in the same manner when unheated; while another serum, which did not protect horse corpuscles when heated, showed no protective power when it was added to the tubes containing horse corpuscles, lecithin, and venom without having previously been heated.

When heated dog serum was substituted for the lecithin in a mixture with guinea-pig corpuscles, rabbit serum showed as much protective power as when lecithin was used.

Rabbit serum which inhibited hemolysis by venom and lecithin, inhibited also the hemolysis by venom and heated horse serum in mixtures with horse, rabbit, and guinea-pig corpuscles, and, as with lecithin, the inhibition was most marked with rabbit corpuscles and least marked with horse corpuscles.

The inhibitory action of rabbit serum was also tested with sodium oleate as an activator. In this experiment, in which guinea-pig corpuscles were used, no inhibitory action was observed.

We tested not only fresh rabbit sera but also rabbit serum on the second as well as the third day after the blood had been withdrawn.

Two sera, which on the first day showed no inhibitory action with horse and guinea-pig corpuscles, were markedly inhibitory on the second day. Two other sera, which on the first day showed slight inhibitory power, remained unchanged in their action on the second day. All rabbit sera, whatever their action may have been on the first day, however, showed distinct inhibitory action on the second day.

On the third day all the sera tested again showed inhibitory action, but while in no case was there an increase, in several cases there appears to have been a decrease of the inhibitory power. The action of such a serum, when tested on three successive days with horse corpuscles, is shown in the following table. In this experiment the same horse corpuscles as well as fresh horse corpuscles were used, the results being the same with the various corpuscles.

Mixture of 0.05 mg. lecithin and 0.01 c.c. venom and 2 c.c. of a 5 per cent suspension of horse corpuscles.

Amt. of rabbit serum.	First day.	Second day.	Third day.
c.c.			
0.6	Total.	0	×
0.4	Total.	0	×
0.2	Total.	0	×××
0.1	Total.	0	Total.
0.05	Total.	×	Total.
....	Total.	Total.	Total.

In certain cases the rabbit serum was reheated on the second and third days, and, in order to determine whether such repeated heating of the serum might influence the inhibitory action, we made parallel experiments with serum which was reheated on the second day and a portion of the same serum only heated on the first day. An experiment was also made with serum reheated on the third day and a portion of the same serum which was not reheated. The results in the two parallel series were similar: the reheated serum, and the serum which was not reheated, acted quantitatively and qualitatively in the same manner, so that it is apparent that the reheating had no influence on the inhibitory power of the rabbit serum.

We may therefore conclude that rabbit serum varies in its inhibitory power; some sera possess no protective power for horse and guinea-pig corpuscles, others show this power slightly, but all show some protective power for rabbit corpuscles. After standing 24 hours all rabbit sera develop protective power for horse and guinea-pig as well as rabbit corpuscles, and after standing 24 hours longer this power may be diminished or remain unchanged.

The serum of a rabbit which had been immunized against heloderma venom was added to mixtures of venom, lecithin, and rabbit corpuscles. We

found that this immune serum inhibited hemolysis in the same degree as did the serum of a normal rabbit; the immune serum had no greater protective power than the normal serum.

We also tested the inhibitory action of the serum of a rabbit that had been immunized against dog serum and dog corpuscles. At the same time parallel experiments were carried out in which normal rabbit serum was used to inhibit hemolysis.

Mixture of venom 0.1 mg. and lecithin 0.05 mg. and 2 c.c. of a 5 per cent suspension of rabbit corpuscles.

Amt. of rabbit serum.	Normal serum.	Serum of rabbit immunized against dog serum and corpuscles.
c.c.		
0.5	X	0
0.3	XX	0
0.1	Total.	X
0.05	Total.	Total.
....	Total.	Total.

In this experiment the serum of the rabbit immunized against dog serum and corpuscles was distinctly more protective for the rabbit corpuscles than the normal serum.

CORPUSCLES OF RABBIT IMMUNIZED AGAINST HELODERMA VENOM.

The corpuscles of a rabbit, immunized against heloderma venom, were washed; a 5 per cent suspension was prepared in the usual manner and the hemolytic influence of venom combined with various activators was tested. At the same time the influence of the venom-activator mixture was tested on corpuscles of normal animals.

When lecithin was added to venom in graded quantities, or when variable quantities of venom were added to lecithin, the corpuscles of the immunized animal showed slightly less hemolysis than those of the normal animal.

Venom 0.1 mg. plus 2 c.c. of a 5 per cent suspension of corpuscles.

Amt. of lecithin.	Normal corpuscles.	Immunized corpuscles.
mg.		
0.05	Total.	Total.
0.03	Total.	XXX
0.01	XXX	XX

Lecithin, 0.05 mg.

Amt. of venom.	Normal corpuscles.	Immunized corpuscles.
mg.		
0.1	Total.	Total.
0.05	Total.	XXX
0.02	XXX	XX

With heated dog-serum as an activator, the corpuscles of the immunized animal showed slightly more hemolysis than the corpuscles of the normal animal; in view of these slight differences and apparent contradictions it seems probable that no marked differences exist between the resistance of the corpuscles of immunized rabbits and of normal rabbits to the hemolytic action of heloderma venom and various activators.

COMPARISON OF THE HEMOLYTIC INFLUENCE OF THE VENOM OF THE HELODERMA SUSPECTUM AND HELODERMA HORRIDUM.

All of the experiments which have been reported so far have been carried out with venom of the *Heloderma suspectum*, but since we were able to obtain venom from the *Heloderma horridum* it appeared advisable to compare the activity of these two venoms. We tested the action of the two venoms combined with lecithin or heated dog serum on guinea-pig, rabbit, and turtle corpuscles. When lecithin was added to the two venoms with all three kinds of corpuscles, slightly more hemolysis was observed in the tubes containing the venom of *Heloderma horridum*. An example of an experiment with turtle corpuscles will serve to show this difference.

1 c.c. of a 5 per cent suspension of turtle corpuscles and lecithin, 0.05 mg.

Amt. of venom.	Heloderma suspectum venom.	Heloderma horridum venom.
c.c.		
0.002	XXX	Total.
0.001	XX	XXX
0.0005	XX	XX

A similar difference was observed when heated dog serum was used as activator; here again the *Heloderma horridum* venom tubes showed slightly more hemolysis.

We also tested the action of heated rabbit and guinea-pig sera as activators for the *Heloderma horridum* venom, with guinea-pig corpuscles.

The *Heloderma horridum* venom was not activated by either of these sera; its behavior was therefore similar to that of the *Heloderma suspectum* venom.

We may conclude that the venom of *Heloderma horridum* and *Heloderma suspectum* are identical in their action.

INFLUENCE OF HEATING ON THE HEMOLYTIC POWER OF HELODERMA VENOM.

It has been shown that heating the venom of various snakes to high temperatures, 100° C., for 30 minutes, does not destroy their hemolytic activity. In the case of the venom of the *Heloderma*, we have shown that boiling does not destroy its toxic property, although it may occasionally lessen it to a very slight extent. In experiments to determine the heat resistance of the hemolytic substance of heloderma venom, fresh venom and dry, dissolved venom were used, with lecithin as an activator. The effect of venom-lecithin mixtures was tested on guinea-pig, *Heloderma*, and turtle corpuscles, and in the main the results were similar with all these corpuscles. The turtle and guinea-pig corpuscles were hemolyzed to the same extent by the various specimens of heated venom, while heloderma corpuscles were more resistant to the heated as well as to the unheated venom.

We tested venom which had been heated to 100° C. for 10 minutes and 30 minutes, and to 120° C. for 15 minutes. Venom which had been heated to 100° C. for 10 minutes was quite as active as unheated venom; when heated to 100° C. for 30 minutes the venom lost most, if not all, of its power to hemolyze the various corpuscles (some variations were observed in individual cases, depending probably upon the susceptibility of the individual corpuscles). When venom was heated to 120° C. all hemolytic power was lost. It should be noted that unheated venom always produced more rapid hemolysis than did the venom which had been heated to 100° C. for 10 minutes.

1 c.c. of 5 per cent suspension of turtle corpuscles and 0.1 mg. of lecithin.

Amt. of venom.	Unheated.	100° C. for 10 minutes.	100° C. for 30 minutes.	120° C. for 15 minutes.
Fresh venom:				
0.03 c.c.	Total.	Total.	××	0
0.02 c.c.	Total.	Total.	××	0
0.01 c.c.	Total.	Total.	××	0
Dry venom:				
0.3 mg.	Total.	Total.	××	0
0.2 mg.	Total.	Total.	××	0
0.1 mg.	Total.	Total.	××	0

The results were in general the same with turtle, heloderma, and guinea-pig corpuscles.

We also compared the influence of heat on the venoms of the *Heloderma horridum* and *Heloderma suspectum*, finding an approximately similar behavior, although the venom of the *Heloderma horridum* was slightly more affected by the heating than the venom of the *Heloderma suspectum;* the two venoms produced the same degree of hemolysis when heated for 10 minutes to 100° C., but after being heated to 100° C. for 30 minutes the venom of *Heloderma horridum* was weaker than that of *suspectum*.

1 c.c. of 5 per cent suspension of rabbit corpuscles and 0.05 mg. of lecithin.

Amt. of venom.	Unheated.	100° C. for 10 minutes.	100° C. for 30 minutes.	120° C. for 15 minutes.
H. suspectum:				
0.3 mg.	Total.	Total.	××	0
0.15 mg.	Total.	Total.	××	0
0.05 mg.	×××	×××	×	0
H. horridum:				
0.3 mg.	Total.	Total.	××	0
0.15 mg.	Total.	Total.	×	0
0.05 mg.	×××	×××	0	0

The action of heat upon the two venoms is therefore in the main the same. The venom of the *Heloderma* is distinctly heat resistant, approximately equally if not more resistant than the hemolysins of certain snake venoms. The hemolytic property of the venom of *Heloderma* is, however, not quite as resistant as the neurotoxic property.

INFLUENCE OF ACIDIFICATION UPON THE HEMOLYTIC ACTION OF HELODERMA VENOM.

A small amount of hydrochloric acid was added to a solution of dry venom which previously gave a neutral reaction, and the hemolysis effected by the acidified and the normal venom was compared. Heated dog serum served as activator. We found no difference in the action of the normal and the acidified venom.

2 c.c. of 5 per cent suspension of dog corpuscles and 0.1 c.c. of dog serum.

Amt. of venom.	Neutral venom.	Acidified venom.
mg.		
1.0	Total.	Total.
0.5	Total.	Total.
0.2	Total.	Total.
0.1	Total.	Total.
0.03	XXX	XXX
0.015	XXX	XXX
0.003	XX	XX

Acidification of the heloderma venom does not interfere with its hemolytic properties.

INFLUENCE OF THE ADDITION OF ALKALI TO HELODERMA VENOM.

Sodium hydroxide and sodium carbonate have been tested as activators for the venom of *Heloderma*. Sodium hydroxide possessed no activating properties. Alone, sodium hydroxide may cause hemolysis of either dog or rabbit corpuscles. When diminishing quantities of a decinormal solution of sodium hydroxide were added to venom, the hemolysis was the same in tubes containing venom and the alkali as in the tubes containing the alkali alone. The results were similar when either dog or rabbit corpuscles were used, except that the rabbit corpuscles were more resistant than the dog corpuscles to the hemolytic influence of the alkali.

1 c.c. of 5 per cent suspension of rabbit corpuscles.

Amt. of N/10 NaOH.	N/10 NaOH.	N/10 NaOH +0.05 mg. venom.
c.c.		
0.5	Total.	Total.
0.2	Total.	Total.
0.1	XX	XX
0.05	0	0
0.025	0	0
0.0125	0	0
0.006	0	0

When 0.1 per cent solution of $NaHCO_3$ was used instead of NaOH, the alkali had not only no activating properties, but the combination of venom with the alkali appeared even to inhibit the hemolysis caused by the alkali alone. Thus 0.4 and 0.2 c.c. of the 0.1 per cent solution of $NaHCO_3$ caused slight hemolysis of dog corpuscles,while similar quantities of alkali combined with 0.5 mg. venom produced no hemolysis. Our experiments were, however,

not sufficiently numerous to establish the latter point, and we can at present only state that alkalis do not activate heloderma venom.

1 c.c. of 5 per cent suspension of dog corpuscles.

Amt. of 0.1 per cent $NaHCO_3$.	0.1 per cent $NaHCO_3$.	0.1 per cent $NaHCO_3$+ 0.5 mg. venom.
c.c.		
0.4	Total.	0
0.2	XX	0

1 c.c. of 5 per cent suspension of guinea-pig corpuscles.

Amt. of 0.1 per cent solution of $NaHCO_3$	0.1 per cent $NaHCO_3$.	0.1 per cent $NaHCO_3$+ 0.5 mg. venom.
c.c.		
0.4	XX	0
0.2	0	0

CONCLUSIONS.

I. Neither the venom of *Heloderma suspectum* nor that of *Heloderma horridum* possesses hemolytic power when added to erythrocytes.

II. When lecithin is added to the venom of either *H. suspectum* or *H. horridum*, erythrocytes are hemolyzed. Horse, ox, sheep, dog, rabbit, guinea-pig, turtle, frog, and heloderma corpuscles may thus be hemolyzed. The quantity of lecithin which must be added to the venom in order to hemolyze the corpuscles varies between one-half and one-tenth of the quantity of lecithin which by itself is able to produce hemolysis. In the experiments with turtle and heloderma corpuscles, a quantity of lecithin equal to $\frac{1}{50}$ and $\frac{1}{400}$ respectively of the hemolytic dose of lecithin is able to activate the venom.

III. Sodium oleate also activates the heloderma venom, but its activating power is less than the power of lecithin.

IV. Alkalis (sodium hydroxide and sodium bicarbonate) do not activate heloderma venom.

V. The blood sera of the horse, dog, and turtle, either unheated or heated to 56° C. or even 70° C., activate the venom of *Heloderma*. On the other hand, ox, sheep, rabbit, guinea-pig, frog, and heloderma serum, either heated or unheated, do not activate the venom.

VI. The blood-corpuscles of *Heloderma*, although not immune against the hemolytic properties of heloderma venom in combination with activators, were occasionally found to be more resistant than other corpuscles tested.

VII. Certain sera, which do not activate the heloderma venom, inhibit the venom-lecithin as well as the venom-serum hemolysis. Heating does not destroy this property. Heloderma serum shows this property more strongly than guinea-pig serum. Rabbit serum varies in its inhibitory power; the serum from one rabbit may inhibit hemolysis, while that of another may not. We found that on the second day after the rabbit serum was separated from the clot its inhibitory power was increased, but on the third day there was again a slight decrease of the inhibitory power. Guinea-pig serum also inhibits venom-sodium-oleate hemolysis, while the rabbit serum tested did not have this inhibiting power. The inhibiting power of the sera varies with the various corpuscles. In the case of the heloderma and rabbit serum, the protective power was greatest in combination with their respective corpuscles.

VIII. Heated horse or turtle serum, both of which activate venom, do not inhibit venom-lecithin hemolysis, but unheated turtle serum may have a slight inhibiting action. The turtle serum contains perhaps both activating and inhibiting substances; in some cases the activating, in other cases the inhibiting substance prevailing.

IX. The corpuscles of a rabbit immunized against heloderma venom were hemolyzed by venom and lecithin as well as by venom and an activating serum; its corpuscles were, however, somewhat more resistant than those of a control rabbit. The serum of a rabbit immunized against heloderma venom does not activate the venom, but inhibits venom-lecithin hemolysis in the same manner as normal rabbit serum.

X. The hemolysins of the venom of *Heloderma* resist heating to 100° C. for 10 minutes. After heating to 100° C. for 30 minutes the hemolytic power of heloderma venom is markedly injured and usually destroyed; after heating to 120° C. for 15 minutes (in the autoclave) the hemolysins are completely destroyed. The heat resistance of the hemolytic substances of heloderma venom is therefore similar to the heat resistance of certain snake venoms and inferior to the heat resistance of the neurotoxic property of the heloderma venom.

XI. The addition of a small amount of acid to the normally neutral venom solution does not change its hemolytic properties.

XII. The venom of *Heloderma suspectum* and *Heloderma horridum* act in the same manner, both being activated by lecithin, or horse or dog serum, and neither possessing the power to hemolyze red blood-corpuscles unless these activating substances are added. They are also equally heat resistant.

XIII. The hemolysins of the heloderma venom differ in certain respects from hemolysins of snake venoms, since they can not be activated by "complements" (such as are contained in guinea-pig serum). It may also be added that, compared with such powerful hemolytic agents as the cobra venom, the venom of *Heloderma* is only weakly hemolytic.

IX.

ACTION OF HELODERMA VENOM ON THE CELLULAR ELEMENTS OF THE BLOOD WITHIN THE LIVING ORGANISM.

By M. K. Meyers and Lucius Tuttle.

14 charts.

ACTION OF HELODERMA VENOM ON THE CELLULAR ELEMENTS OF THE BLOOD WITHIN THE LIVING ORGANISM.

By M. K. Meyers and Lucius Tuttle.

There is a paucity of information regarding the reaction of the cellular elements of the blood in response to the presence within the living body of a venom. The only reference to the subject we could find in the literature concerned work done by Chateney,* who found in an animal rendered immune to a lethal dose of cobra venom a rise in the leucocytes from 6,500 to 8,000; three control rabbits that had received lethal doses of the venom died, showing a hypoleucocytosis. Although Calmette† states, presumably on the authority of Chateney, that injection of a venom in amount sufficient to cause feeble intoxication is followed by a hyperleucocytosis, it will be seen that the above rise in the number of leucocytes is present only in a certain number of cases.

The following experiments prove definitely that leucocytosis is one of the reactions of the organism against the introduction of a specific venom; and it would seem that in an animal already immune this leucocytosis is greatly increased.

The dried venom was dissolved in a small quantity of salt solution and injected subcutaneously, unless otherwise stated. In estimating hemoglobin the Fleischl-Miescher instrument was employed.

The accompanying protocols and charts are more or less self-explanatory.

Experiment.	Day.	Time.	Red blood corpuscles.	11b. per 100 c.c.	White blood corpuscles.	Weight of rabbit.	Amt. of venom injected.	Remarks.
				gms.		*gms.*	*gm.*	
Rabbit I, expt. 1....	Nov. 19	11h 35m a.m.	7,300,000	9.70	7,206	1,600	0.005	
		11 45 a.m.	....	....	....	....	....	Venom injected.
		12 00 noon	6,600,000	14.30	5,400	....	....	
		1 00 p.m.	6,990,000	13.28	6,170	....	....	
		3 00 p.m.	....	....	....	....	....	Rabbit fed.
		5 00 p.m.	6,430,000	14.82	11,500	....	....	
	Nov. 20	10 00 a.m.	6,750,000	11.24	18,000	....	....	Differential count
	Nov. 21	10 00 a.m.	5,136,000	7.82	11,900	....	....	taken; leucocytosis.
Rabbit I, expt. 2....	Nov. 25	11 00 a.m.	5,560,000	8.16	12.700	1,500	0.006	
		11 45 a.m.	....	....	....	....	....	Venom injected.
		1 30 p.m.	6,240,000	11 71	25,400	....	....	Leucocytosis.
		4 00 p.m.	4,160,000	12.78	10,400	....	....	
	Nov. 26	10 30 a.m.	4,540,000	9.18	15,000	....	....	
	Nov. 27	10 30 a.m.	4,970,000	10.22	8,600	....	....	

*Chateney. Les réactions leucocytairea vis-à-vis de certaines toxines. Thèse de Paria, 1894.

†Calmette. Les venins, les animaux venimeux et la sérothérapie antivenimeuse. Paris, 1907, pp. 226, 227.

Experimeat.	Day.	Time.	Red blood corpuscles.	Hb. per 100 c.c.	White blood corpuscles.	Weight of rabbit.	Amt. of venom injected.	Remarks.
				gms.		*gms.*	*gm.*	
Rabbit I, expt. 3....	Dec. 2	10^{h} 50^{m} a.m.	4,710,000	9 18	10,800	1,480	0.0075	
		11 30 a.m.	....	....	....	....	....	Venom injected.
		12 00 noon	5,760,000	9.69	7,900	....	....	
		2 30 p.m.	5,320,000	13.40	5,200	....	....	
	Dec. 3	9 30 a.m	5,140,000	9.69	14,100	....	....	
Rabbit I, expt. 4....	Dec. 12	11 15 a.m.	4,500,000	8.93	10,600	1,400	0.0075	
		11 40 a.m.	....	....	....	....	....	Do.
		12 00 noon	6,760,000	10.71	11,000	....	....	
		12 45 p.m.	5,930,000	....	6,600	....	....	
	Dec. 14	10 30 a.m.	4,500,000	8.92	9,600	....	....	
Rabbit II, expt. 1....	Nov. 22	11 15 a.m.	5,200,000	5.10	11,700	1,700	0.005	
		12 00 noon	....	....	....	....	....	
		12 15 p.m.	6,200,000	7.64	9,500	....	....	Do.
		1 15 p.m.	4,470,000	7.64	11,665	....	....	
		5 00 p.m.	5,700,000	8.16	24,500	....	....	Leucocytosis.
	Nov. 23	11 15 a.m.	5,780,000	4 86	12,500	....	....	
Rabbit II, expt. 2....	Nov. 25	11 15 a.m.	....	3.70	6,993	1,560	0.006	
		11 45 a.m.	....	....	....	....	....	Venom injected.
		4 00 p.m.	5,310,000	8 +	11,107	....	....	
	Nov. 26	10 30 a.m.	3,760,000	7.92	17,520	....	....	
Rabbit II, expt. 3....	Dec. 2	10 50 a.m.	4,410,000	8.16	12,240	1,480	0.0075	
		11 30 a.m.	....	....	....	....	....	Do.
		12 00 noon	5,710,000	9.18	5,920	....	....	
		4 30 p.m.	3,440,000	....	....	....	....	
	Dec. 3	2 15 p.m.	4,190,000	7.48	14,640	....	....	
Rabbit III	Nov. 29	10 45 a.m.	4,860,000	7.14	9,100	1,720	0.015	
		11 00 a.m.	....	....	....	....	....	Do.
		11 30 a.m.	5,420,000	12 26	9,900	....	....	
		12 15 p.m.	6,720,000	7.65	9,600	....	....	
		2 30 p.m.	6,700,000	10.88	8,200	....	....	
		4 00 p.m.	....	13.29	....	....	....	
	Nov. 30	10 00 a.m.	4,770,000	7.14	12,500	....	....	
Rabbit IV.....	Nov. 30	10 30 a.m.	5,050,000	8.16	12,560	1,600	0.005	
		11 30 a.m.	....	....	....	....	....	Do.
		12 00 noon	5,220,000	4 76	9,500	....	....	
		2 15 p.m.	5,420,000	8.67	11,500	....	....	
		4 00 p.m.	5,700,000	....	7,440	....	....	
	Dec. 1	9 45 a.m	4,380,000	7 14	9,400	....	....	
Rabbit V, expt. 1....	Dec. 5	10 00 a.m.	6,370,000	10.92	4,500	1,440	0.020	
		10 30 a.m.	....	....	....	....	....	Do.
		11 00 a.m.	5,800,000	15.35	7,680	....	....	
		12 00 noon	6,250,000	14.66	5,100	....	....	
		2 00 p.m.	8,000,000	14.02	7,600	....	....	
	Dec. 6	11 15 a.m.	5,140,000	12.75	7,500	....	....	
	Dec. 7	10 15 a.m.	5,000,000	10.20	11,000	....	....	
Rabbit V, expt. 2....	Dec. 11	10 00 a.m	5,330,000	9.56	7,600	1,420	0 020	
		10 30 a.m.	4,070,000	10.20	6,700	....	....	Do.
		10 45 a.m.	7,000,000	8.92	2,300	....	....	
		2 00 p.m.	7,000,000	....	....	....	....	Rabbit died.
Rabbit VI, expt. 1...	Dec. 5	10 20 a.m.	5,260,000	13.29	6,200	1,540	0.020	
		10 30 a.m.	....	....	....	....	....	Venom injected.
		11 00 a.m.	7,720,000	17 37	6,240	....	....	
		12 00 noon	7,040,000	13 29	5,440	....	....	
	Dec. 6	11 00 a.m.	....	13.29	11,120	....	....	
	Dec. 7	2 15 p.m.	....	....	10,960	....	....	
Rabbit VI, expt. 2...	Dec. 11	10 00 a.m.	....	12.78	13,640	....	0.020	
		10 20 a.m.	....	....	....	....	....	Do.
		11 00 a.m.	5,570,000	10.71	12,080	....	....	
		12 00 noon	4,860,000	13 29	11,520	....	....	
	Dec 12	9 45 a.m.	5,820,000	10 29	7,800	....	....	
Rabbit VII.....	Dec. 16	10 30 a.m.	3,230,000	7.01	7,600	2,600	0.0075	
		11 00 a.m.	4,670,000	....	7,920	....	....	Do.
		12 15 p.m	5,350,000	10.21	13,360	....	....	
		2 45 p.m.	4,600,000	8.92	9,400	....	....	
	Dec. 17	10 15 a.m.	4,500,000	9.35	8,000	....	....	
Rabbit VIII.....	Dec. 19	11 00 a.m	4,800,000	4.46	14,000	1,440	0 0075	
	Dec. 21	10 15 a.m	4,660,000	9.56	14,000	....	....	
		10 30 a.m.	....	....	....	....	....	Do.
		10 45 a.m	4,830,000	11 48	7,200	....	....	
		11 45 a.m.	....	10.20	4,900	....	....	
		4 00 p.m.	5,880,000	6.34	16,500	....	....	
	Dec. 22	10 30 a.m.	4,060,000	7.00	11,500	....	....	
	Dec. 23	10 25 a.m.	4,100,000	7.44	8,400	....	....	
Rabbit IX.....	Dec. 23	11 20 a.m.	3,160,000	5.52	3,800	1,940	0.0075	
		11 45 a.m.	....	....	....	....	....	Do.
		12 15 a.m.	2,980,000	8.16	3,200	....	....	
		1 00 p.m.	3,200,000	7.82	3,600	....	....	
		2 30 p.m.	3,200,000	8.45	3,900	....	....	Rabbit died shortly after 2^{h} 30^{m} p.m.

Experiment.	Day.	Time.	Red blood corpuscles.	Hb. per 100 c.c.	White blood corpuscles.	Weight of rabbit.	Amt. of venom injected.	Remarks.
				gms.		*gms.*	*gm.*	
Rabbit X.....	Jan. 4	10h 40m a.m.	4,200,000	9.18	7,400	2,160	0.0275	
		10 45 a.m.	....	....	....	....	....	Venom injected.
		10 55 a.m	6,060,000	9.18	7,600	....	....	
		11 15 a.m.	4,700,000	9.35	3,700	....	....	
		12 10 p.m.	5,530,000	10.20	6,200	....	....	
		2 30 p.m.	4,800,000	10.65	4,800	....	....	
Rabbit XI.....	Jan. 15	11 00 a.m.	....	....	8,500	1,880	0.00564	
		11 30 a.m.	....	....	....	....	....	Do.
		11 50 a.m.	....	....	6,400	....	....	
		12 35 a.m.	....	....	13,200	....	....	
		1 30 p.m.	....	....	3,600	....	....	
Rabbit XII.....	Jaa. 17	9 50 a.m.	....	....	3,300	1,500	0.0045	
		10 00 a.m.	....	....	....	....	....	Do.
		10 25 a.m.	....	....	3,800	....	....	
		10 50 a.m.	....	....	3,500	....	....	
		11 25 a.m.	....	....	10,100	....	....	
		10 50 a.m.	....	....	2,400	....	....	
		3 00 p.m.	....	....	5,200	....	....	
Rabbit XIII.....	Dec 27	12 00 noon	4,890,000	10.65	13,700	1,640	0.010	Mesenteric vein.
		12 00 noon	4,950,000	11.24	22,320	....	....	Ear vein.
		12 10 p.m.	....	....	....	....	....	Venom injected.
		2 00 p.m.	7,200,000	14 98	9,400	....	....	Mesenteric vein.
		2 00 p.m	4,275,000	7.44	....	....	....	Ear vein.
		2 00 p.m.	....	....	....	....	....	Rabbit died shortly after 2 p.m.
Rabbit XIV.....	Dec. 30	11 00 a.m.	4,770,000	9 35	16,100	1,900	0.010	Mesenteric vein.
		11 00 a.m.	4,490,000	9 35	15,000	....	....	Ear vein.
		11 30 a.m.	....	....	....	....	....	Venom injected.
		2 40 p.m.	6,130,000	7 22	9,000	....	....	Mesenteric vein.
		2 40 p.m.	....	10.96	15,900	....	....	Ear vein.
Rabbit XV.....	Jan. 8	11 15 a.m	5,100,000	8.2	6,200	1,310	0.0076	Mesenteric vein.
		11 15 a.m.	5,200,000	9.5	22,800	....	....	Ear vein.
		11 32 a.m.	....	....	....	....	....	Venom injected.
		12 15 p.m.	5,200,000	8.4	4,700	....	....	Mesenteric vein.
		12 15 p.m.	5,700,000	10.00	3,400	....	....	Ear vein.
		2 20 p.m.	4,900,000	8 40	4,600	....	....	Mesenteric vein.
		2 20 p.m.	5,440,000	8 40	9,600	....	....	Ear vein.
	Jan. 9	10 30 a.m.	5,200,000	7.60	4,000	....	....	Mesenteric vein.
		10 30 a.m.	5,020,000	13.80	6,320	....	....	Ear vein.
Rabbit XVI.....	Jan. 24	9 45 a.m.	....	....	9,800	1,750	0.00525	Do
		10 10 a.m.	....	....	....	....	....	Venom injected.
		10 40 a.m.	....	....	19,100	....	....	Ear vein; leucocytosis.
		10 55 a.m.	....	....	14,000	....	....	Ear vein.
		11 18 a.m.	....	....	3,000	....	....	Mesenteric vein.
		11 22 a.m.	....	....	7,700	....	....	Splenic vein.
		11 23 a.m.	....	....	16,600	....	....	Ear vein.
		11 26 a.m.	....	....	7,000	....	....	Vena cava.
Rabbit XVII, expt.1.	Jan. 25	11 45 a.m.	4,000,000	7.52	14,200	1,480	0.055	Venom injected.
		12 15 p.m.	4,080,000	8 69	11,200	....	....	
		2 10 p.m.	4,100,000	6.63	22,000	....	....	Leucocytosis.
		4 30 p.m.	....	....	270,000	....	....	Do.
	Jan. 26	9 45 a.m.	....	....	40,300	....	....	Do.
		3 00 p.m.	....	....	8,100	....	....	
	Jan. 27	8 45 a.m.	....	....	10,600	....	....	
	Jan. 28	8 45 a.m.	....	....	12,500	....	....	
	Jan. 30	8 45 a.m.	....	....	7,400	....	....	
	Jan. 31	11 45 a.m.	....	....	11,800	....	....	
Rabbit XVII, expt 2.	Jan. 31	11 45 a.m.	....	....	11,800	1,510	0.060	
		1 00 p.m.	....	....	....	....	....	Venom injected.
		1 30 p.m.	....	....	7,200	....	....	
		3 00 p.m.	....	....	7,000	....	....	
	Feb. 1	9 15 a.m.	....	....	9,800	....	....	
		11 00 a.m.	....	....	15,000	....	....	
		11 15 a.m.	....	....	24,000	....	....	Leucocytosis.
		12 30 p.m.	....	....	36,200	....	....	Do.
		2 45 p.m.	....	....	44,000	....	....	Do.
		4 45 p.m.	....	....	64,000	....	....	Do.
	Feb. 2	9 00 a.m.	....	....	52,000	....	....	Do.
		1 55 p.m.	....	....	15,200	....	....	Do.
	Feb. 3	9 00 a.m.	....	....	19,000	....	....	
Rabbit XVII, expt.3.	Feb. 11	9 30 a.m.	....	....	....	1,420	0.070	Veaom injected.
		10 30 a.m.	....	....	2,800	....	....	
	Feb. 12	9 10 a.m.	....	....	9,000	....	....	
	Feb. 13	9 00 a.m.	....	....	10,600	....	....	
	Feb. 14	8 50 a.m.	....	....	9,500	....	....	
	Feb. 15	8 40 a.m.	....	....	9,100	....	....	

Experiment.	Day.	Time.	Red blood corpuscles.	Hb. per 100 c.c.	White blood corpuscles.	Weight of rabbit.	Amt. of venom injected.	Remarks.
				gms.		*gms.*	*gm.*	
Rabbit XVII, expt. 4	Feb. 18	$5^h\ 00^m$ p.m.	....	....	....	1.640	0.080	
	Feb. 19	11 00 a.m.	....	....	13,200	....	....	
		3 00 p.m.	....	....	3,900	....	....	
	Feb 20	10 00 a.m.	....	....	9,800	....	....	
		3 00 p.m.	....	....	15,000	....	....	
	Feb. 21	9 20 a.m.	....	....	18,000	....	....	
		2 20 p.m.	....	....	18,500	....	....	
	Feb. 22	8 40 a.m.	....	....	8,000	....	....	
	Feb. 24	9 20 a.m.	....	....	8,300	....	....	
Dog I	Jan. 31	10 30 a.m.	6,400,000	10.54	13,000	4,820	0.0289	Venom injected, half given subcutaneously at $11^h\ 10^m$ a.m., half given intravenously by saphenous vein at $11^h\ 30^m$ a.m.
		12 15 p.m.	6,130,000	14 05	25,200	....	....	Leucocytosis.
		2 15 p.m.	4,430,000	12.77	24,000	....	....	Do.
		4 10 p.m.	8,100,000	18.90	53,000	....	....	Do.
	Feb. 1	11 45 a.m.	5,800,000	13.62	35,000	....	....	Do.
Dog II	Feb. 6	9 30 a.m.	6,660,000	17.04	19,700	7,760	0.0466	6 mg. per kilo.
		11 18 a.m.	....	....	....	....	....	Venom injected intravenously.
		11 58 a.m.	6,700,000	17.37	16,000	....	....	
		1 00 p.m.	7,830,000	20 43	24,200	....	....	
		2 30 p.m.	....	19.76	30,480	....	....	Leucocytosis.
		3 30 p.m.	7,550,000	....	....	....	....	
		4 15 p.m.	7,900,000	19.92	20,000	....	....	
	Feb. 8	9 40 a.m	7,000,000	19.41	32,000	....	....	Do.

DIFFERENTIAL COUNTS.

	Per cent.	No.
Blood of rabbit I at period of greatest leucocyte count, 18,000:		
Amphophiles	69.6	12,528
Lymphocytes	25.8	4,644
Mononuclears	2.4	432
Transitional	1.0	180
Basophiles	1.2	216
Eosinophiles	0.0	0
Total	.....	18,000
Blood of dog I before injection, 13,000:		
Neutrophiles	77.0	10,020
Lymphocytes	12.0	1,560
Mononuclears	5.6	728
Eosinophiles	4.8	624
Basophiles	0.0	0
(?)	0.6	78
Total	.....	13,010
At period of highest leucocytosis:		
Neutrophiles	94.6	50,140
Lymphocytes	3.4	1,803
Mononuclears	0.8	423
Eosinophiles	0.8	423
Basophiles	0.0	0
(?)	0.4	212
Total	.....	53,001

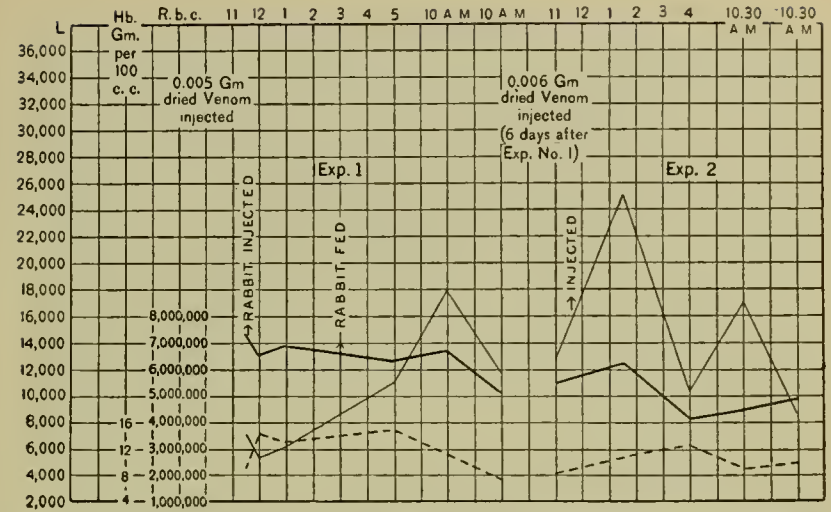

FIG. 25.—*Rabbit* 1, *Experiment* 1. The morning of the day after the injection the number of leucocytes reaches 18,000; which is a distinct rise in the number of leucocytes in this rabbit. A differential count shows that polymorphonuclear amphophilic leucocytes constitute 69.6 per cent of the total number. This is rather high. The amount of hemoglobin and number of red cells run in fairly parallel curves.

Rabbit 1, *Experiment* 2. The number of leucocytes rises immediately after the injection to 25,400, then falls; the next morning the number is again high. Variations in the hemoglobin index seem to fluctuate about a nearly constant mean.

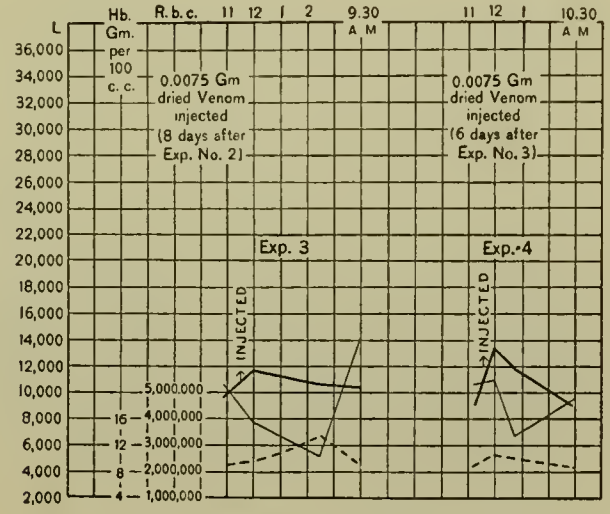

FIG. 26.—*Rabbit* 1, *Experiment* 3. No leucocytosis detected.
Experiment 4. No leucocytosis detected.

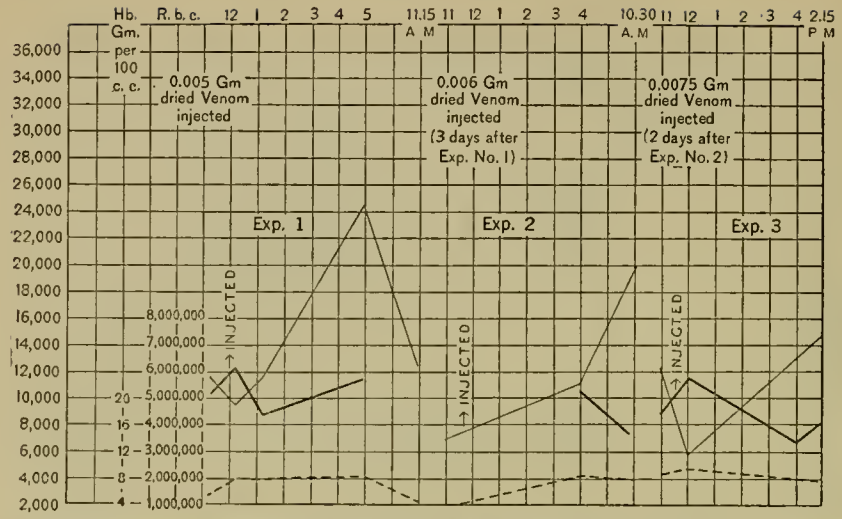

FIG. 27.—*Rabbit* 2, *Experiment* 1. Rise in number of leucocytes 5 hours after injection; followed next morning by an absence of leucocytosis.
Experiment 2. The morning after injection number of leucocytes is high.
Experiment 3. The morning after injection number of leucocytes is higher than before injection.

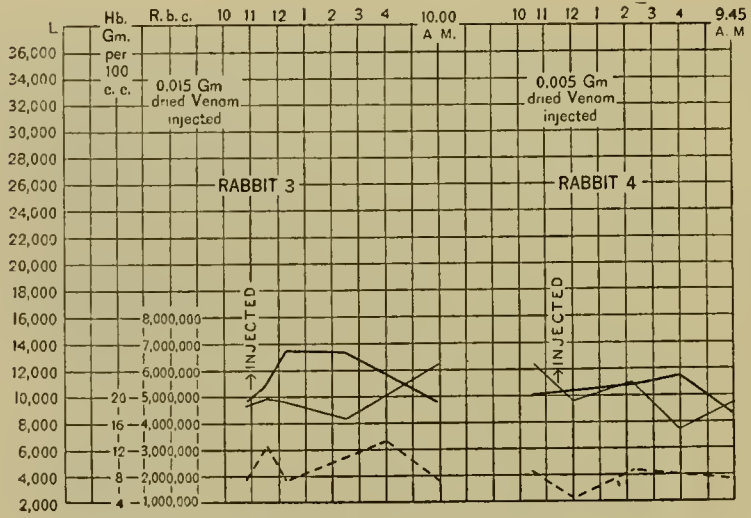

FIG. 28.—*Rabbit* 3. Only very slight rise found on morning of day following injection.
Rabbit 4. No leucocytosis.

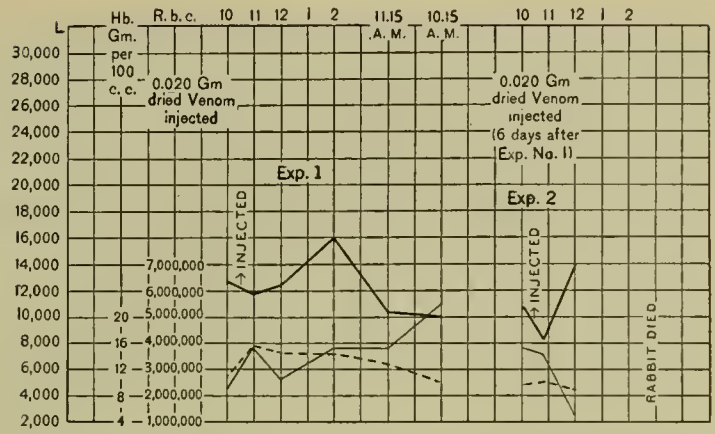

FIG. 29.—*Rabbit* 5, *Experiment* 1. A rise to over 10,000 leucocytes occurred 2 days after injection of a relatively large amount of venom. The animal had a low leucocyte count at beginning of experiment.

Experiment 2. A large quantity of venom was again injected; number of leucocytes fell, and 3 or 4 hours after injection rabbit died. Slight rise in number of leucocytes occurred after first injection, followed by a distinct fall after second injection of venom.

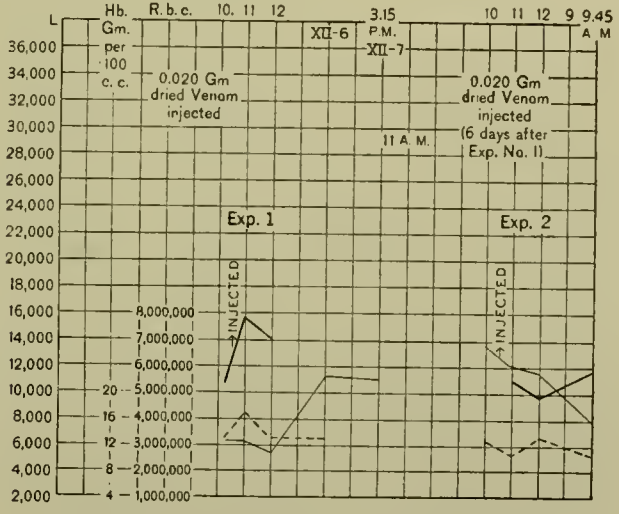

FIG. 30.—*Rabbit* 6, *Experiments* 1 *and* 2. Large doses of venom used.

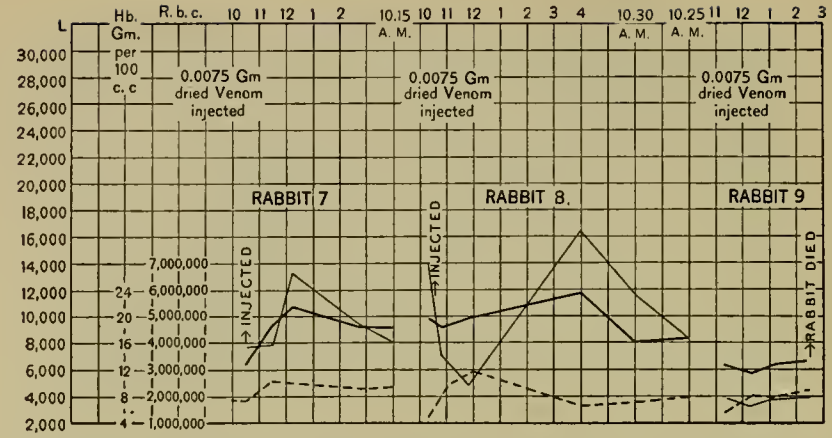

FIG. 31.—*Rabbits 7 and 8.* Smaller doses of venom were used here than in experiments on rabbit 6; a slight rise in both occurred. In rabbit 8 the rise is preceded by a fall.
Rabbit 9. This rabbit had an initially low leucocyte count; it succumbed to a rather small dose of venom. No rise in number of leucocytes.

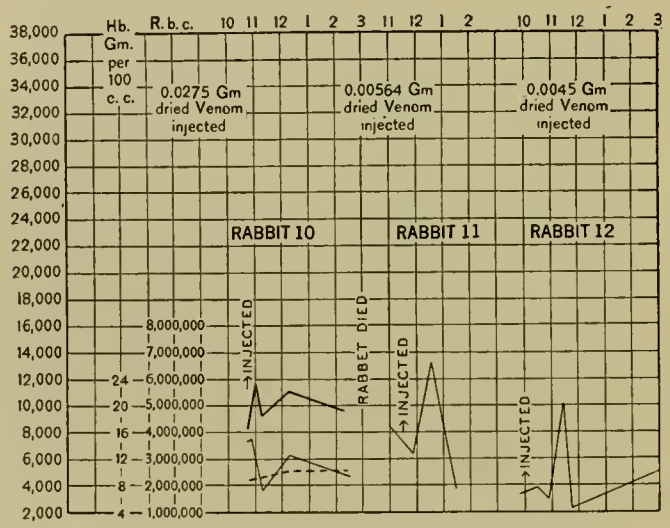

FIG. 32.—*Rabbit 10.* Large dose; no leucocytosis. Some initial fall in number of leucocytes.
Rabbit 11. Slight fall followed by a not considerable rise in leucocytes.
Rabbit 12. Small number of leucocytes observed in beginning of experiment; followed by inconsiderable rise after injection.

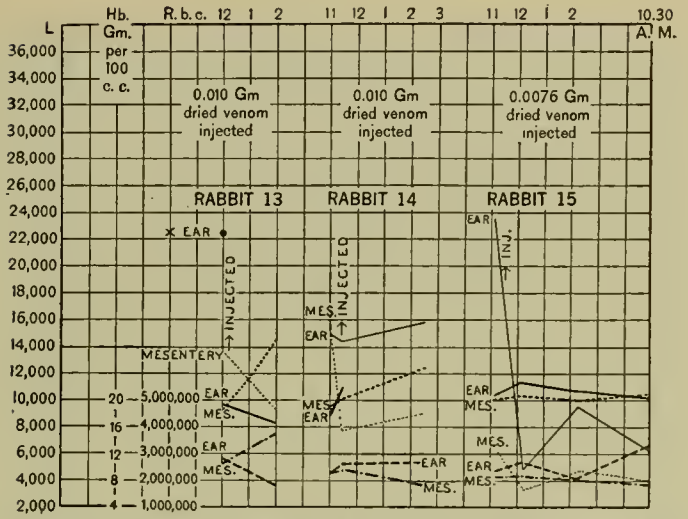

FIG. 33.—*Rabbit* 13. Simultaneous counts were made from a vein of the ear and one of the mesentery; that of the ear was high, that of the mesentery lower; the count of the mesenteric vein fell after venom had been injected. *Rabbit* 14. An initial high leucocyte count was found in both the blood from the ear and the mesenteric vein. After injection no striking rise in the leucocytes from the ear-vein was noted, and there was a marked fall in the number of leucocytes in the blood of the mesenteric vein, followed by a slight rise. *Rabbit* 15. A very marked fall in number of leucocytes was observed in blood of ear-vein, associated with a slight fall in number in mesenteric vein of a rabbit that had a preexisting leucocytosis following injection of a relatively small dose of venom.

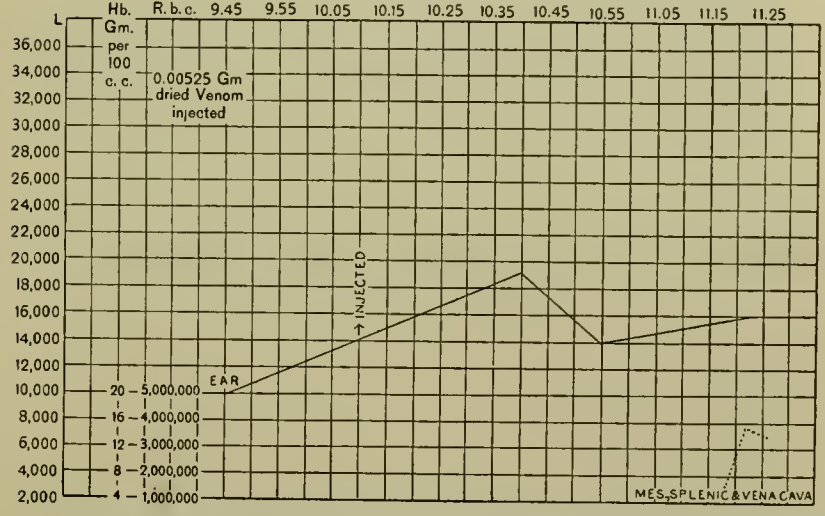

FIG. 34.—*Rabbit* 16. A rise in leucocytes of blood of ear-vein was found after injection of venom, with a considerably lower number of leucocytes in successive counts taken from veins of abdominal cavity (mesenteric vein, splenic vein, vena-cava). In this experiment an initial fall in number of leucocytes could be excluded through repetition of early counts.

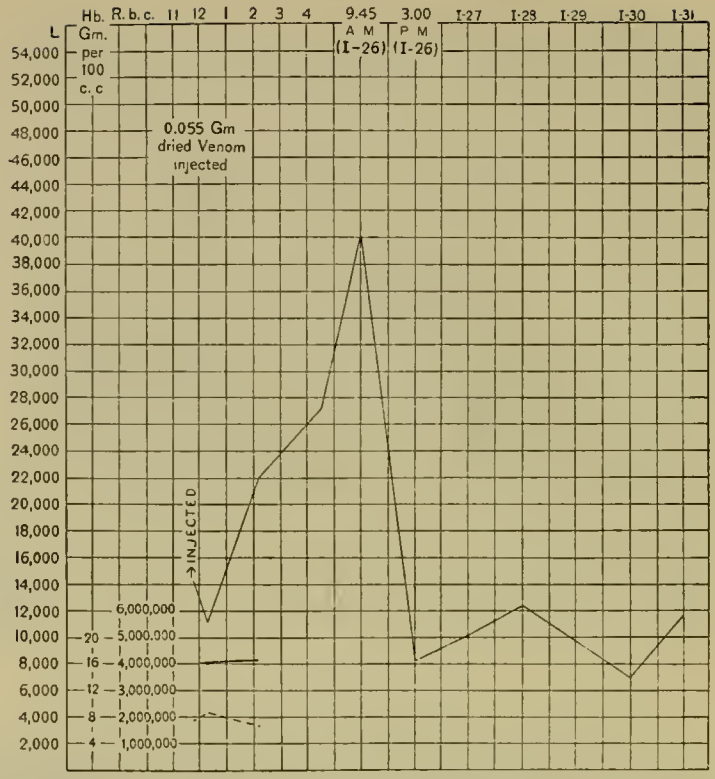

FIG. 35.—*Rabbit* 17, *Experiment* 1. This rabbit had been immunized against venom by injection of increasing doses. Figure shows very marked leucocytosis following administration of very large dose of venom that persisted about a day. The rabbit had a relatively high leucocyte count at beginning of experiment.

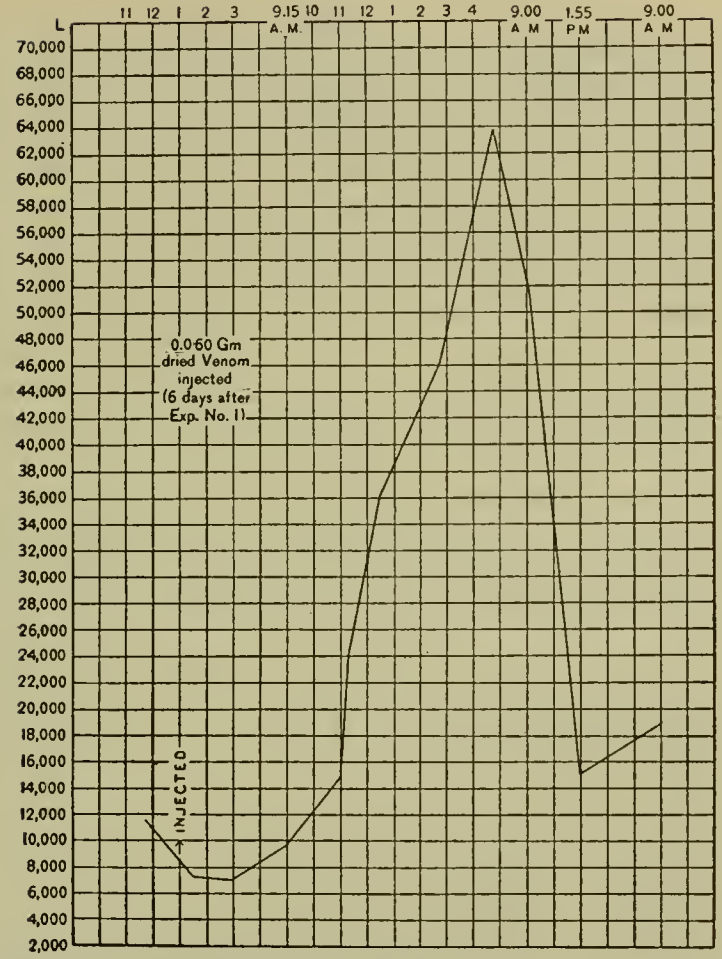

FIG. 36.—*Rabbit* 17, *Experiment* 2. With only slightly larger dose of venom than in preceding experiment leucocytosis was much higher, but latent period was longer than after preceding injection. In experiments 1 and 2 the rabbit had a relatively high leucocyte count at beginning of experiment.

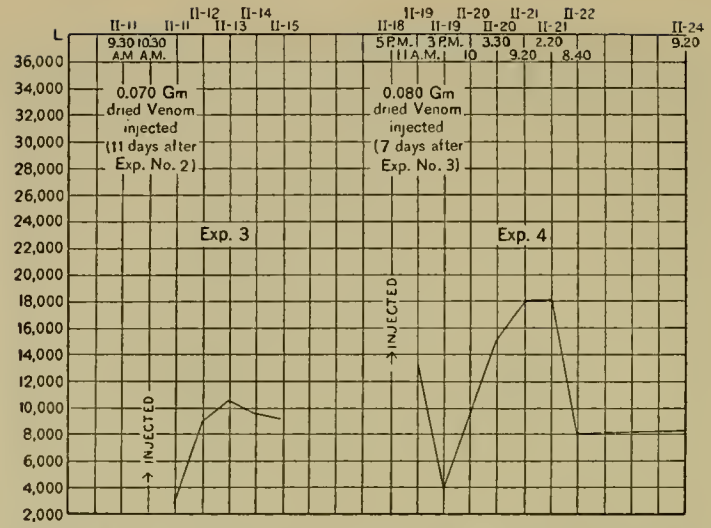

FIG. 37.—*Rabbit* 17, *Experiment* 3. Amount of venom slightly larger than in preceding experiment; if there was a leucocytosis, it occurred at times other than when counts were made, 1 and 2 days respectively after injection.

Rabbit 17, *Experiment* 4. Amount of venom administered again increased. Slight rise in number of leucocytes on third day after injection was probably not due to action of venom. It is difficult to explain why no leucocytosis was found in this case; it may have occurred during the night.

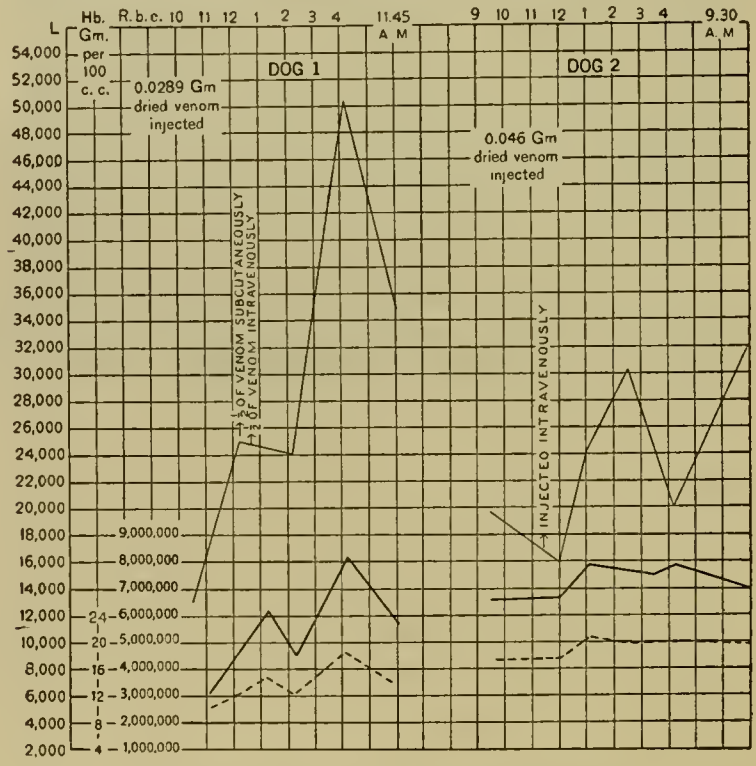

FIG. 38.—*Dog* 1. Leucocytosis followed injection of venom, part of which was given intravenously.
Dog 2. Leucocytosis followed intravenous administration of venom.

SUMMARY.

The subcutaneous injection of solutions of heloderma venom in quantities varying from 5 to 27.5 mg. into a series of 16 non-immunized, apparently healthy rabbits of an average weight of 1,500 grams, led in many cases to a rise in the number of leucocytes within the 24 hours following the injection. This rise in the number of leucocytes was frequently preceded by a slight fall. This rise in the number of leucocytes was not noticeable in all cases; it was absent in several cases, in which relatively large quantities of venom had been injected. It was also absent in three cases in which the animals died a few hours after the injection. In one of these rabbits the injection was followed by a marked fall in the number of leucocytes.

In two healthy, non-immunized dogs, non-lethal doses of venom administered partly or entirely intravenously were followed by a leucocytosis that was noticeable as early as 2 hours after the injection and persisted at least a day. The increase in the number of leucocytes was in all cases due to an increase in the polymorphonuclear neutrophiles or pseudoeosinophiles. In contradistinction to ordinary rabbits, a rabbit that had gained a certain degree of active immunity through frequently repeated injections of gradually increasing doses of venom showed a very marked leucocytosis following the successive injections of 55 and 60 mg. respectively of the venom. In this animal there seems to been a slight but constant increase in the number of leucocytes. After the injection of 55 mg., an increase in the number of leucocytes appeared 2 hours 30 minutes after the injection, 4 hours 45 minutes after the injection the increase was greater, and was very high 22 hours after the injection. After administration of 60 mg. of venom, the leucocytosis was observed a day after the injection and seemed to persist about 24 hours. On two later occasions, when large doses of the venom (70 and 80 mg. respectively) were given, repeated examinations of the blood during the succeeding 4 days did not show the presence of a leucocytosis. There can be little doubt that the injection of large doses of venom during the process of immunization increased very markedly the leucocytosis following the administration of venom.

Whether this leucocytosis depends on an actual increase in the number of leucocytes or on an unequal distribution of leucocytes in the various regions of the body must at present be left undecided.

Injection of venom into rabbits or dogs has no marked effect on the number of erythrocytes or quantity of hemoglobin in the circulating blood.

X.

THE INFLUENCE OF HELODERMA VENOM UPON PHAGOCYTOSIS.

By Lucius Tuttle.

THE INFLUENCE OF HELODERMA VENOM UPON PHAGOCYTOSIS.

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We have tested the influence of the venom of *Heloderma* upon the production of a cellular exudate in the peritoneal cavity of the guinea-pig, upon the phagocytosis of red corpuscles of the pigeon in the peritoneal cavity of a guinea-pig, and also upon the phagocytosis of bacteria *in vitro* by the leucocytes of the dog in vitro.

The influence of venom upon the production of cellular exudate was studied in the peritoneal cavity of 18 guinea-pigs. On the first day of the experiment we injected sterile bouillon into the peritoneal cavity of the guinea-pigs; on the following day, approximately 24 hours after the injection of the bouillon, we injected into the peritoneal cavities of half of the guinea-pigs small quantities of venom, usually 4 mg. pro kilo. of body-weight. The other guinea-pigs received no injection on the second day, except in a few cases in which we injected 0.85 per cent sodium-chloride solution. At different times, from 1 to 5 hours after the injection of the venom and 25 to 29 hours after the injection of bouillon, the fluid was drawn from the peritoneal cavity, smears were made, stained, and examined microscopically.

We found the same characteristic exudate in all cases, both in those in which venom had been injected and in those in which no venom was injected. This peritoneal exudate was composed mostly of polymorphonuclear leucocytes, with a few mononuclear leucocytes. The venom therefore seems to exert no influence on the formation of cellular peritoneal exudate.

In the next experiments we injected venom (4 mg. pro kilo.) and pigeon's red corpuscles into the peritoneal cavities of guinea-pigs, making several injections before removing the peritoneal fluid for microscopic examinations. On two or three successive days we injected venom, and each injection of venom was immediately followed by an injection of defibrinated pigeon's blood. In the majority of experiments we injected the venom and pigeon blood on two successive days, and on the third day venom alone. At various periods from 1 to 24 hours after the last injection of venom, fluid was removed from the peritoneal cavity and examined microscopically. Control experiments were made in which pigeon erythrocytes, but no venom, was injected.

In studying the smears of the peritoneal fluid, we noted the number of phagocytes containing pigeon-blood corpuscles, the number of pigeon erythrocytes lying free in the fluid, the number of guinea-pig erythrocytes, and the amount of granular detritus.

Usually there was little granular detritus, which bore no relation to the phagocytosis and varied from time to time in the various individuals. No relation existed between the amount of granular detritus and the injection or non-injection of venom. A few guinea-pig blood corpuscles were observed in the peritoneal fluid in every case, but in no case was any large number found.

In certain experiments, no pigeon corpuscles were found in the smears. We have not included these experiments in our series. When venom was injected into the peritoneal cavities of guinea-pigs, we found marked or moderate phagocytosis in 15 out of 19 cases, while, when no venom was injected, we found similar conditions in 19 out of 23 cases. It thus appears that the degree of phagocytosis does not differ materially after the injection of venom.

In one series of these experiments we counted the number of phagocytic cells containing few pigeon corpuscles and the number containing many corpuscles. In each animal of the control as well as of the venom series 100 mononuclear leucocytes were classified in each case.

Percentage of mononuclear leucocytes containing pigeon corpuscles.

Animals injected with venom.			Animals used as controls.		
No. of phagocytes.	With many corpuscles.	With few corpuscles.	No. of phagocytes.	With many corpuscles.	With few corpuscles.
	p. ct.	*p. ct.*		*p. ct.*	*p. ct.*
1	13	42	1	16	47
2	7	39	2	9	28
3	12	44	3	14	33
4	18	36	4	12	35
5	11	27	5	17	39
Average	13	36	Average	12	37

From this table it will be seen that the leucocytes of the animals which received venom took up the pigeon's corpuscles in almost exactly the same manner as did the corpuscles of the normal animals.

Proportion of leucocytes containing few and many bacteria per hundred leucocytes not containing any bacteria.

Amount of venom.	Bacteria with venom.			Bacteria without venom.		
	None.	Few.	Many.	None.	Few.	Many.
10 mg. (0.5 per cent solution of venom)	100	115	26	100	63	25
	100	31	8	100	55	23
	100	69	16	100	67	16
7 mg.	100	110	50	100	60	15
	100	127	45	100	56	19
	100	131	62	100	88	19
5 mg.	100	69	22	100	94	15
	100	70	30	100	106	16
	100	125	27	100	64	11

The influence of venom on phagocytosis was tried *in vitro* with the corpuscles of a dog. These corpuscles were obtained by injecting a suspension of aleuronat into the pleural cavity of a dog. The dog was killed about 36 hours after the injection and the pleural exudate removed. To 1 c.c. of this exudate

containing mostly polynuclear leucocytes we added 0.5 c.c. of a 24-hour bouillon culture of a staphylococcus and to five tubes, 10, 7, 5, 2, or 1 mg. of venom dissolved in 0.85 per cent NaCl solution, while to five control tubes equal quantities of salt solution were added. These tubes were placed in an incubator and smears were made and examined 2, 4, and 18 hours later.

We carried out four experiments with the exudate of 4 dogs. No difference was found between the degree of phagocytosis in most cases. In one experiment the leucocytes in the tubes containing 7 and 5 mg. had taken up fewer bacteria than their controls, but in all other experiments neither 10, 7, nor 5 mg. of venom influenced the phagocytosis.

We may conclude that the venom in no way prevents the phagocytosis of bacteria. The wide variations observed in these experiments are probably due to individual variations.

CONCLUSIONS.

The intraperitoneal injection of heloderma venom into guinea-pigs does not modify the cellular exudate resulting from the intraperitoneal injection of sterile bouillon.

The injection of heloderma venom into the peritoneal cavity of guinea-pigs in no way interferes with the phagocytosis of pigeon's blood.

The addition of heloderma venom to mixtures of dog's leucocytes and staphylococci *in vitro* does not inhibit phagocytosis.

It is therefore apparent that heloderma venom has no injurious influence on leucocytes and that it does not diminish the phagocytic activity of the leucocytes.

XI.

CAN THE PRESENCE OF ANTIBODIES TO THE VENOM OF HELODERMA SUSPECTUM BE DEMONSTRATED IN THE BLOOD SERUM OF THIS ANIMAL BY THE METHOD OF COMPLEMENT FIXATION?

By Ellen P. Corson-White.

CAN THE PRESENCE OF ANTIBODIES TO THE VENOM OF HELODERMA SUSPECTUM BE DEMONSTRATED IN THE BLOOD SERUM OF THIS ANIMAL BY THE METHOD OF COMPLEMENT FIXATION?

By Ellen P. Corson-White.

An animal secreting a poison deadly to itself would be a rare instance of the preservation of a factor detrimental to the individual and to the race. Many stories of poisonous snakes were found in olden times and out of their mists Francisco Redi (1)* first noted the fact that poisonous snakes were harmless to themselves. Fontana (2, 3) later gave experimental proof of this immunity by forcing vipers to bite themselves or each other. Phisalix and Bertrand (4) confirmed Fontana, and were able to show that harmless reptiles, while less resistant than poisonous snakes, would withstand doses of venom fatal to other animals. Weir Mitchell (5) had previously demonstrated the same fact in cases of the rattlesnake, both when bitten and when the venom was injected subcutaneously or intravenously. Since these observations, auto-immunity has been described in scorpions (6), vipers, black snakes, cobras, and in practically all poisonous snakes. Fayrer (7), in his study of Indian snakes, says that a serpent not only is not poisoned by its own venom, nor even with that of another individual of its own species, but is only slightly susceptible to the venom of another species. Its venom, however, often kills an innocuous reptile; the smaller this serpent and the less poisonous it is, the more readily it succumbs to the poison. Cooke and Loeb (8) have found this same protective power in the Gila monster. Natural immunity, while generally present in snakes, is not absolute (9), and can be overcome by sufficiently large doses of venom (10).

What gives this immunity to snakes? Phisalix and Bertrand (11) thought that the protective power was due to the presence of venom, which entered the blood as an internal secretion from the venom glands of the upper jaw. These glands have been described by many observers—Fontana (2), Leydig (12), Reicht (13), Blanchard (14), Jourdain (15), etc.—in both harmless and poisonous snakes.

Phisalix found that the blood of many snakes was distinctly toxic (16); if injected subcutaneously or intraperitoneally it produced local and general effects very similar to that produced by venom. These observers removed (17) the poison glands from 46 vipers and 167 days later injected the blood

*The figures in parentheses refer to the Bibliography on page 198.

from the viper into guinea-pigs. All these guinea-pigs survived, while all the control animals injected with the blood of normal vipers died. The toxicity of the blood is destroyed by heating it to 68° C., but if the heated blood is injected into guinea-pigs it confers an increased resistance to venom.

Calmette (18) also observed this and called attention to the fact that while the toxicity of blood was lost at 68° C., the venom would resist prolonged heating at much higher temperatures. He concludes that the toxic substance is probably not venom but a diastatic substance, physiologically distinct, but analogous to some constituent of the venom. It may be a forerunner from which the venom is produced in the process of secretion by the poison gland.

Flexner and Noguchi (19, 20) made no definite statement concerning this fact, but distinguished between the two sets of active principles by their capability to unite with or be activated by their homologous and heterogeneous complements.

Fraser (21) thinks the immunity depends on changes, similar to artificial immunization, produced in the serum by constant absorption of venom, which all snakes secrete in varying amounts. The venom may enter the body through abrasions, through the mucous membrane of the alimentary tract, or may be absorbed directly from the venom gland by its lymph channels. The animal (4) may also receive venom from its own bite or the bite of other animals. In the artificially immunized animal, definitely antitoxic substances may be demonstrated by the usual methods. Metschnikoff (22), by mixing the blood-serum and venom of a scorpion, showed an undoubted neutralizing power in the blood.

Fraser (2) found that the serum of a *Hamadryas* destroyed the toxic action of cobra venom; this power was present both when the blood-serum and venom were injected simultaneously or at intervals of 15 or 30 minutes. This fact also he found was true of the blood serum of the Australian blacksnake and the venom of the same snake. His experiments show that definite substances are present in the serum of poisonous snakes antidotal against their own or alien venom. Natural antidotal substances are, however, not as strong as those found in the blood of animals immunized against venom.

In the case of the *Heloderma suspectum*, Cooke and Loeb showed that while the monster possessed an immunity against its venom, its blood was not toxic, nor did it possess antidotal properties. Notwithstanding this deficiency in demonstrable antitoxin the serum might perhaps contain certain antibodies which could be demonstrated by the complement fixation test of Bordet and Gengou (23), especially inasmuch as Cooke and Loeb (8) have shown that the venom contains an antigen which on injection into rabbits causes the formation of precipitins.

Two series of experiments were made to test for the presence of antibodies in the blood-serum or the power of the mixture of serum and venom to fix complement. In one, the active and the inactive serum of the *Heloderma* was used with an antihuman hemolytic system and in the other inactive serum and an antisheep system.

A preliminary titration of the serum was made with both hemolytic systems to determine the amount of complement fixed by the serum alone. Guinea-pig serum was used for the complement and such dilutions of this serum were made that 0.1 c.c. of the dilution would equal respectively 0.01, 0.02, 0.03 c.c., etc., up to 0.1 c.c.

Active heloderma serum to the amount of 0.5 c.c. was incubated for 2 hours with 0.1 c.c. of each dilution of complement and likewise two other sets, II and III, of 0.5 c.c. of inactive sera with 0.1 c.c. of each dilution of guinea-pig serum. At the expiration of this time, to the first series was added 1 c.c. of 5 per cent suspension of washed human cells and 2 units of antihuman amboceptor, to series II the same was added, and to series III, 1 c.c. of 5 per cent washed sheep-cells suspension and 2 units of antisheep amboceptor. After a second incubation of an hour the result was read. In all, three separate titrations of the serum were made with the different systems. The complement present in the unheated serum activated the amboceptor and in every tube there was complete hemolysis. The result with the inactive serum and the two hemolytic systems were practically the same.

(1) 0.5 c.c. 1–3000 dilution of venom and .05 c.c. complement.
(2) 0.5 c.c. 1–6000 dilution of venom and .05 c.c. complement.
(3) 0.5 c.c. 1–12000 dilution of venom and .05 c.c. complement.
(4) 0.5 c.c. 1–24000 dilution of venom and .05 c.c. complement.
(5) 0.5 c.c. 1–48000 dilution of venom and .05 c.c. complement.
(6) 0.5 c.c. 1–64000 dilution of venom and .05 c.c. complement.
(7) 0.5 c.c. 1–80000 dilution of venom and .05 c.c. complement.
(8) 0.5 c.c. 1–96000 dilution of venom and .05 c.c. complement.
(9) 0.5 c.c. 1–128000 dilution of venom and .05 c.c. complement.

Incubate for 2 hours at 37° C., then add 1 c.c. washed erythrocytes and 2 units of amboceptor to every tube and again incubate for 1 hour at 37° C.

Result:
(1) No hemolysis.
(2) No hemolysis.
(3) No hemolysis.
(4) No hemolysis.
(5) No hemolysis.

Result:
(6) Partial hemolysis.
(7) Partial hemolysis.
(8) Complete hemolysis.
(9) Complete hemolysis.

In the three titrations using the sheep system the dose absorbed was between 0.05 + 0.06 c.c. of complement.

Three different titrations with the two systems were then made, using a fixed dose of 0.05 c.c. of complement and 0.5 c.c. of varying dilutions of the venom.

Another titration was now made of the venom in dilutions between 40,000 and 90,000, fixing the dose causing almost complete hemolysis at 1 to 70,000.

Having decided on the amount of complement fixed by the serum and venom, four series of fixation tests were made with the two hemolytic systems, using in every case a complement 18 hours old and made up of the blood of three guinea-pigs. 0.5 c.c. of serum was added to 0.5 c.c. of 1 to 70,000 dilution of venom and the sum of the amounts of complement absorbed by each factor alone added; that is, 0.05 c.c. + 0.05 c.c. or 0.1 c.c. of complement; con-

trolling this were similar tubes with increasing and decreasing doses of complement.

(1) 0.5 c.c. serum and 0.5 c.c. venom and .15 c.c. complement.
(2) 0.5 c.c. serum and 0.5 c.c. venom and .12 c.c. complement.
(3) 0.5 c.c. serum and 0.5 c.c. venom and .10 c.c. complement.
(4) 0.5 c.c. serum and 0.5 c.c. venom and .08 c.c. complement.
(5) 0.5 c.c. serum and 0.5 c.c. venom and .06 c.c. complement.

Incubate for 2 hours at 37° C., then add 1 c.c. washed erythrocytes and 2 units of amboceptor and incubate again for 1 hour at 37° C.

Result:
(1) Complete hemolysis.
(2) Complete hemolysis.
(3) Partial hemolysis.

Result:
(4) No hemolysis.
(5) No hemolysis.

CONCLUSIONS.

In all four tests made and using both systems, no true fixation of complement could be determined. In no case was more complement absorbed than the sum of that fixed by the venom and serum alone. By this method, therefore, the presence of an antibody can not be demonstrated.

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XII.

ACTION OF CALMETTE'S COBRA ANTIVENIN UPON THE VENOM OF HELODERMA.

By Moyer S. Fleisher and Leo Loeb.

ACTION OF CALMETTE'S COBRA ANTIVENIN UPON THE VENOM OF HELODERMA.

By Moyer S. Fleisher and Leo Loeb.

The question whether the antivenin prepared by Calmette through injection of cobra venom into horses exerts any influence upon the venom of *Heloderma* seemed to us of sufficient interest to warrant the carrying out of some experiments in this direction.

The majority of the investigators in the field of snake venoms have come to the conclusion that antivenins have a definite specific relation to the venom which served as antigen. This specific relation is of twofold character. In the first place, the venoms of various snakes contain apparently several poisonous substances, which exert a totally different pharmacologic action, as, for instance, the neurotoxin, the hemorrhagin, and the substance causing thrombosis. The chemical relationship of these substances is uncertain at present, and very divergent opinions concerning it have been expressed.

As far as the immunizing power of snake-venom antisera is concerned, all investigators admit that an antivenin prepared from a venom that contains only neurotoxin is powerless against the hemorrhagin or coagulin, which are contained principally or side by side with neurotoxin in various venoms. Sera prepared from hemorrhagins as antigen do not neutralize the neurotoxin of snake venoms.

There exists, however, possibly a second kind of specificity. Are the neurotoxins of the various snake venoms identical, or do they differ among each other? The same question may be asked in regard to the hemorrhagins. Here also the majority of investigators believe that there is a decided specificity of the various neurotoxins or hemorrhagins, and consequently a specificity of the antivenins prepared with certain neurotoxins or hemorrhagins. This view is to a great extent based upon the results obtained by Lamb and is also upheld by Noguchi. According to this view the antivenin prepared by the injection of cobra neurotoxin protects only against cobra neurotoxin, or perhaps, though very much less, against the neurotoxin of very nearly related snakes, while it is powerless against all other neurotoxins. Calmette, on the other hand, maintains that the neurotoxins of the various snake venoms are identical and that the antivenin prepared with cobra venom as antigen protects against the neurotoxins of other snake venoms.

Under these conditions it was of interest to determine whether the cobra antivenin possesses any protecting influence against heloderma venom. Heloderma venom exerts its lethal influence exclusively through a neurotoxin; from a pharmocologic point of view it resembles closely cobra venom, although

it is not so poisonous as the latter, if equal amounts of the mixture which is called venom are compared. Cobra antivenin should therefore exert a powerful protecting influence against the venom of heloderma. On the other hand, from the point of view of zoological relationship, *Heloderma* is further distant from the *Cobra* than any snake. According to the prevalent view cobra antivenin should be without any effect on the venom of heloderma.

In our experiments we used a cobra antivenin which Doctor Calmette kindly put at our disposal; 1 c.c. of this antivenin was sufficient to neutralize 1 mg. of cobra venom (tested by Doctor Calmette); 0.01 mg. of cobra venom is a dose lethal for a white mouse; 1 c.c. of Calmette's serum neutralizes, therefore, 100 lethal doses. We used white mice in our experiments. In all cases a definite quantity of dry venom and Calmette's serum were mixed *in vitro*. The mixture, having stood 45 minutes at room temperature, was injected into the mice subcutaneously. In control experiments mice were injected with mixtures of venom and 0.85 per cent NaCl solution, or rabbit serum, horse serum, and diphtheria antitoxin. None of these latter substances exerted any protecting influence. In another experiment less than a lethal dose of heloderma venom was used (namely, 0.1 mg.), and it was found that rabbit serum did not increase the toxicity of heloderma venom. Cobra antivenin, on the other hand, had a definite though very slight antitoxic effect on heloderma venom; it is able to neutralize a fraction of a lethal dose. After the addition of cobra antivenin a certain number of mice died as quickly under the influence of heloderma venom as the control mice injected with venom plus 0.85 per cent NaCl solution. In each experiment there were, however, others in which the antivenin delayed the appearance of the heloderma-venom poisoning; 40 per cent of the animals injected with one or two lethal doses of heloderma venom mixed with either 0.5 or 0.75 c.c. of cobra antivenin survived, while all the controls died. If more than one or two lethal doses of heloderma venom were used, 0.5 c.c. of cobra antivenin was without effect. In an experiment in which the antivenin and the heloderma venom were injected at different places without having been previously mixed in the test-tube, merely a delay in the lethal action was noticeable.

In an experiment the mixture was kept in the thermostat, and the venom underwent some deterioration, in consequence of which some of the control mice survived. This experiment is, therefore, inconclusive.

Although the action of Calmette's cobra antivenin on heloderma venom is very slight, the serum neutralizing only a fraction of a lethal dose, there can be no doubt that it does exist. The same result was obtained in several experiments, while horse and rabbit serum and the diphtheria antitoxin (namely, the dissolved globulins) were without effect. We can not hold an unknown accidental factor responsible for these results.

We conclude then that Calmette's cobra antivenin has a slight neutralizing action on the venom of *Heloderma*. The action is, however, several hundred times weaker than on cobra venom. Our experiments prove, therefore, the existence of a relative specificity of the snake-venom antitoxins, prepared with

the neurotoxins as antigen, the antitoxin acting strongly on the neurotoxin used as an antigen, and only very weakly upon the neurotoxin of a related reptile.

The recent experiment of Bang and Overton,* who found that the cobra antitoxin had a protecting influence on tadpoles which were kept in a solution of crotalus venom, to which the cobra antivenin had been added, seems to point to a similar conclusion as that reached by ourselves. We must take into consideration the fact that Bang and Overton observed the neutralizing effect in experiments with crotalus venom, a substance rich in hemorrhagin; but perhaps in this case also the protecting influence of the antivenin was mainly directed against the neurotoxin in the crotalus venom.

The following table gives a summary of our result:

Fluids injected.	Mice used.	Died.	Survived.	Mice used as controls.
Calmette serum 0.5 c.c.. venom 0.15 mg	12	7	4	4
Calmette serum 0.5 c.c.. venom 0.3 mg	4	3	1	2
Calmette serum 0.5 c.c., venom 0.6 mg	1	1	0	1
Calmette serum 0.5 c.c., venom 1.2 mg	1	1	0	1
Calmette serum 0.5 c.c., venom 1.8 mg	1	1	0	0
Calmette serum 0.5 c.c., venom 2.25 mg	1	1	0	0
Calmette serum 0.75 c.c., venom 0.14 mg.; mixture kept at room temperature	4	2	2	4
Calmette serum 0.75 c.c., venom 0.14 mg.; mixture kept at 37.5° C	4	1	3	†4
Calmette serum 0.5 c.c., venom 0.15 mg.; injected separately	2	2	0	1
Horse serum 0.75 c.c.; venom 0.14 mg	6	6	0	4
Diphtheria antitoxin 0.5 c.c., venom 0.15 mg	2	2	0	1
Rabbit serum 0.5 c.c.; venom 0.15 mg	2	2	0	1
Rabbit serum 1.0 c.c., venom 0.15 mg	2	2	0	1
Rabbit serum 0.75 c.c.; venom 0.1 mg.; 3 affected as soon as controls; 3 survived (1 control died, 1 survived)	2	2	0	1

*Bang and Overton. Biochem. Zeitschrift, Bd. 34, p. 428, 1911.

†Only one of these died.

XIII.

INFLUENCE OF VARIOUS STAINS UPON VENOM.

By Lucius Tuttle.

INFLUENCE OF VARIOUS STAINS UPON VENOM.

By Lucius Tuttle.

In the following experiments we have determined the influence of heat upon venom, both before and after the addition of certain stains. We have tested the influence of these stains upon the toxicity as well as upon the production of precipitates. In further experiments we have tested the influence of light upon venom mixed with various stains, comparing these specimens with similar ones which had been left in darkness.

INFLUENCE OF STAINS AND HEAT UPON VENOM.

In most of these experiments we used a 0.5 per cent solution of eosin (G. Grübler, water-soluble) made up with 0.85 per cent sodium-chloride solution, but in a few cases we used a 0.5 per cent solution of neutral red made up with the same diluent. In experiments testing the toxicity of the various solutions we injected quantities of the various mixtures into mice, in one experiment only (an experiment in which eosin was used) we used rats to test the toxicity of the venom. In all cases we heated the venom for 10 minutes to a temperature of 100° C.

We found that when 0.15 c.c. of the 0.5 per cent eosin solution was added to 0.85 c.c. of diluted venom (0.5 c.c. of venom diluted with twice its volume of 0.85 per cent NaCl solution plus 0.35 c.c. of NaCl solution) no precipitate appeared, but if only 0.1 c.c. of the eosin solution was added to 0.9 c.c. diluted venom (0.5 c.c. of diluted venom and 0.4 c.c. NaCl solution) a precipitate appeared after boiling for 10 minutes. We furthermore noted that when sufficiently large quantities of 0.5 per cent eosin solution were added to fresh undiluted, unfiltered venom, which as usual was turbid, it was possible to diminish but not to prevent altogether the precipitate. Even though we added a quantity of solid eosin more than sufficient to saturate the undiluted unfiltered venom (the solid eosin being dissolved on heating) we were unable to prevent precipitates in this turbid venom. Aron found previously that addition of eosin to a proteid solution prevented the formation of precipitates.*

With the neutral red we were unable to prevent precipitation even when 0.3 c.c. was added to 0.7 c.c. of diluted venom (0.5 c.c. of venom diluted with twice its volume of 0.85 per cent NaCl solution and 0.2 c.c. NaCl solution).

We tested the influence of eosin and heat combined upon the venom in five experiments. In each series we injected several mice with different quantities of venom which had been heated, venom mixed with eosin and heated, and

*Aron. Biochemische Zeitschrift, 1907, v, 413.

venom which had been mixed with eosin but had not been heated. It has been previously shown that heloderma venom may be heated to 100° C. without seriously impairing its toxicity.

In four of the five experiments the animals (in three cases mice, in one case rats) injected with unheated venom mixed with eosin died most rapidly; those injected with venom which had been heated died less rapidly than those injected with the venom mixed with eosin and heated. In one experiment the degree of toxicity of the various venom mixtures was practically the same, independent of whether eosin had or had not been added or whether or not they had been heated. In this experiment the venom was heated to 100° C. for 10 minutes and also to 120° C. for 10 minutes. In spite of the negative result of this one experiment it would appear that the addition of eosin to heloderma venom prevents the slight harmful influence of heat upon the toxicity of the venom, perhaps by diminishing the precipitate which includes a part of the venom.

Neutral red we found prevented neither heat coagulation nor the slight destructive influence of heat upon the heloderma venom. On the contrary, the addition of neutral red to venom, even if the mixture was not heated, appeared to lessen the toxicity. We found that mice injected with either heated or unheated venom died about 50 minutes after the injection, while mice injected with either heated or unheated venom to which neutral red had been added died about 1 hour 30 minutes after injection. We have, however, carried out too few experiments with neutral red to enable us to draw any definite conclusion as to whether this stain diminishes the toxicity of heloderma venom or not.

COMBINED INFLUENCE OF LIGHT OR DARKNESS AND VARIOUS STAINS ON VENOM.

In testing the influence of light or darkness upon mixtures of solutions of venom and various stains we have used eosin, 1: 500, 1: 2000, and 1: 5000 and methylene blue 1: 500 and 1: 5000. The test-tubes containing the venom were exposed to the diffuse daylight of the room for several days and the controls were wrapped in black cloth and kept in a drawer. We have tested the toxicity of the solutions, both those left in the light and those left in the dark, after 2, 4, and 14 or 18 days.

In every experiment we used fresh heloderma venom diluted with twice its volume of 0.85 per cent sodium-chloride solution and sterilized. To small quantities of this sterilized solution equal quantities of a previously sterilized solution of the stain were added and the tubes placed in previously sterilized tubes, closed with cotton, and sealed with paraffin. In every case duplicate sets of tubes were mixed, so that one might be left in the light and the other in the dark.

We found no difference between the action of the various dilutions of the same stain. Thus, the action of the 1: 500 solution was the same as of either the 1: 2000 solution or the 1: 5000 solution of eosin. Therefore, in describing the results of these experiments, we shall describe only the experiments carried out with the 1: 500 eosin solution.

We found in the majority of experiments that two days after mixing the eosin and venom the venom left in the dark had lost little if any of the toxicity when tested by injections into mice. The venom which had been exposed to the light, however, had lost a considerable part of its toxicity; when a quantity equal to one lethal dose was injected, the animal usually survived; frequently even the injection of a quantity equal to two and a half lethal doses produced no effect, but when a quantity equal to five lethal doses was injected the mice died.

Four days after the venom and eosin had been mixed, we occasionally noted a weakening of the venom which had been left in the darkness; the toxicity was not lost, but the mice lived longer after the injection. In most cases, however, no change was noted. The venom which had been exposed to the light had lost somewhat more of its toxicity; when a quantity equal to two and a half lethal doses was injected the mice did not die, but succumbed when a quantity equal to five lethal doses was injected.

After 14 or 18 days we usually found no change in the venom which had been left in the dark, while the mixture of venom and eosin which had been exposed to the light was not toxic for mice, even though a quantity equal to five lethal doses was injected. The two different dilutions of methylene blue, like the various dilutions of eosin, both acted in the same manner.

Two days after mixing the venom and methylene blue the specimens exposed to light were not lethal for mice, though a quantity equal to one lethal dose was injected; at this time the injection of two and a half lethal doses of the venom-methylene-blue solution exposed to light was able to cause the death of the mouse. The mixtures which had been left in the dark had lost none of their activity.

After four days no further change was noted. The injection of two and a half lethal doses of the venom-methylene-blue solution exposed to light was still lethal for mice.

After 14 or 18 days the mixtures left in the dark had lost little if any of their toxic properties. The mixtures left in the light had lost their toxicity and the injection of a quantity of venom-methylene-blue solution which had been exposed to the light, equal to five lethal doses, did not have any effect upon mice.

Noguchi* found that eosin affects the hemolytic and toxic properties of cobra, daboia, and rattlesnake venom when these are mixed with the stain and exposed to sunlight for 30 hours. The destructive action was most marked when the photodynamic action of eosin was tested with rattlesnake and least marked when tested with cobra venom. Indeed, rattlesnake venom lost almost entirely its toxic properties after 8 hours' exposure to the photodynamic action of eosin. Erythrosin possessed a slightly destructive action on the toxins of these same snake venoms, but its action was much weaker than that of eosin.

It appears that these venoms react to the photodynamic activity of eosin in much the same manner as they react to heat. Rattlesnake venom, which is

*Noguchi. Jour. Exper. Med., 1906, VIII, 252.

more easily destroyed by heat than cobra venom, is also more rapidly and fully destroyed by the photodynamic action of eosin.

The toxins of heloderma venom, which are relatively thermostable, are not easily destroyed by the photodynamic activity of eosin, since it requires several days for the eosin to destroy the toxic properties of this venom.

SUMMARY.

1. The addition of 0.5 per cent solution of eosin to freshly diluted and filtered venom in the proportion of 0.15 c.c. of eosin solution to 0.85 c.c. venom-sodium-chloride solution prevents the formation of a coagulum even when the venom is heated to 100° C. for 10 minutes.

2. The addition of neutral red to venom does not prevent the formation of a coagulum.

3. The addition of eosin to venom prevents the slight decrease in the toxicity of venom usually resulting from heating the venom to 100° C. It is probable that neutral red has no similar action.

4. The addition of eosin (1: 500, 1: 2000, or 1: 5000) or of methylene blue (1: 500, or 1: 5000) to fresh diluted venom does not influence the toxicity of such venom even after a period of 14 or 18 days if the venom is kept in the dark.

5. If, however, eosin (1: 500, 1: 2000, or 1: 5000) or methylene blue (1: 500 or 1: 5000) be added to venom and this mixture be exposed to the light, the venom gradually loses its toxic properties and after 14 or 18 days seems to have lost all toxicity.

XIV.

ADSORPTION OF HELODERMA VENOM BY SUSPENSIONS OF VARIOUS SUBSTANCES.

By Moyer S. Fleisher and Leo Loeb.

ADSORPTION OF HELODERMA VENOM BY SUSPENSIONS OF VARIOUS SUBSTANCES.

By Moyer S. Fleisher and Loe Loeb.

We have investigated the adsorptive power of various organic and inorganic substances for heloderma venom; and furthermore, we have determined whether the various organs of one species and the corresponding organs of animals of different species showed a specific power of adsorption for the venom of *Heloderma*.

TECHNIQUE.

In determining the power of adsorption of certain organic and inorganic substances and of the organs of various animals for the venom of *Heloderma*, we used both diluted fresh and dissolved dry venom. Whenever the fresh venom was used, 1 c.c. of the venom was added to 9 c.c. of 0.85 per cent sodium-chloride solution; the dried venom was ground in a mortar to very fine powder and 0.85 per cent sodium-chloride solution was added, in such quantity that each cubic centimeter of fluid contained 1 mg. of venom. The solutions of both fresh and dried venom were always filtered before the addition of the material whose adsorptive power was to be tested.

In most cases we used 7 c.c. of venom solution and a quantity equal to one-fourteenth part of the solution of the various adsorbent substances, usually 0.5 c.c. A special note is added in those experiments in which the bulk of the substances added to the venom solution varied. The venom solution and the adsorbent substances were mixed in a mortar in order to obtain an even suspension. This suspension, in a small, tightly stoppered bottle, was then placed in a shaker for 2 hours 30 minutes. At the end of this time the mixtures were either filtered through filter paper or centrifugated in order to separate the adsorbent material from the fluid part of the mixture. In our earlier experiments all mixtures were filtered; on account of the fact that a certain amount of the fluid was held back by the filter paper and because of the length of time required for the filtration; this method was discarded and in all our later experiments the centrifuge was used to throw down the adsorbent particles from the suspension. In a few experiments even prolonged centrifugation did not separate the solution and the suspended particles, and these mixtures were filtered through Berkefeld filters.

Throughout our experiments mice were used to test the toxicity of the supernatant fluid and of the residue.* When the fresh diluted venom was used

*The layer which we call residue contained the coarser particles of the adsorbent material.

for testing the adsorptive power of the various substances, we injected in each mouse quantities of the supernatant fluid which should have contained between 0.050 c.c. and 0.005 c.c. of venom, provided no venom had been adsorbed by the substance tested; it having been shown that the smallest of these doses of venom was sufficient to kill a mouse.

In the case of dissolved dry venom we injected either 2 c.c., 1 c.c., or 0.5 c.c. of the solution after separation from the adsorbent particles. The smallest dose injected should, therefore, have contained 0.5 mg. of venom, which is approximately $3\frac{1}{3}$ times the lethal dose.

In the first experiments we used the fresh venom; but in all our later work the dissolved dry venom, which was more uniform in its action, was employed.

In testing the toxicity of the residue, we used that portion of the mixtures which had been thrown down by the centrifuge after the supernatant fluid had been decanted (or in the case of oils, the surface layer). This residue was shaken up with 0.85 per cent sodium-chloride solution and again centrifuged. After the second centrifugation the supernatant fluid containing the venom which had perhaps been retained between the adsorbent particles, was poured off and the residue added to 7 c.c. of salt solution. Thus the residue was suspended in a quantity of non-toxic fluid equal to the original venom solution, and any toxic effect of this emulsion could justly be ascribed to the venom adsorbed by the particles. In each case 2 c.c. of this emulsion corresponding to $13\frac{1}{3}$ lethal doses, provided the venom had been entirely adsorbed, were injected into mice.

In each series of experiments some control mice were injected with the same quantities of the venom solution.

ADSORPTIVE POWER OF CARMINE.

The adsorptive power of carmine was tested with fresh diluted venom. Venom solution, mixed with a quantity of carmine equal to half its volume, entirely lost its toxic action. Three mice injected with quantities of the supernatant fluid, which, had no venom been adsorbed, should have contained from one to ten lethal doses of venom, survived the injection. In one experiment the venom solution mixed with a quantity of carmine equal to one-quarter of its volume was injected into two mice in quantities corresponding to two and six lethal doses respectively, and both survived the injection, although both were slightly affected. In another experiment the supernatant fluid derived from a mixture of carmine and only one-eighth of its volume of venom solution was injected into mice in quantities corresponding to a little more than two and six lethal doses. The first survived the injection and the second died.

According to these experiments, carmine, when present in sufficient quantity, adsorbs the heloderma venom completely or almost completely. A quantity of carmine equal to one-eighth of the volume of the venom solution adsorbs more than half of the venom.

ADSORPTION OF VENOM BY ANIMAL CHARCOAL.

In testing the adsorption of venom by animal charcoal, both fresh and dry venom were used. In the case of the fresh venom we added quantities of charcoal equal to one-half, one-quarter, and one-sixteenth of the volume of the venom solution and found, after these mixtures had been shaken for 2 hours 30 minutes, that all the mice injected with the supernatant fluid survived the injection. In an experiment in which a quantity of charcoal equal to only one thirty-second of the volume of the venom solution had been added, the adsorption was incomplete, and two mice injected with the supernatant fluid from this mixture died as soon as their respective controls. Quantities of charcoal equal to one-sixteenth of the volume of venom solution are, therefore, sufficient to adsorb all or almost all of the venom; while a quantity of the charcoal equal to one thirty-second of the volume of the venom solution adsorbs less than half of the venom.

In experiments in which the time of contact between the venom and the charcoal was shortened to 15 minutes, the adsorption was not complete and animals injected with two or four lethal doses of venom died. Throughout subsequent experiments we used solutions of dry venom with a quantity of charcoal equal to one-sixteenth of the volume of the solution. Only one mouse of the sixteen injected with quantities of this supernatant fluid died as a result of the injection, even when amounts corresponding to $13\frac{1}{3}$, $6\frac{2}{3}$, and $3\frac{1}{3}$ were administered. This mouse injected with a quantity of the fluid corresponding to $6\frac{2}{3}$ lethal doses lived, however, longer than the control mouse, which had received a similar quantity of the pure venom solution.

Charcoal not only adsorbs all, or almost all, of the venom, but moreover it holds the adsorbed venom very firmly. Eight mice injected with 2 c.c. of a suspension containing the residue of the charcoal-venom mixture survived the injection. It is not probable that the venom was removed from the charcoal during the washing with salt solution.

The addition of 2 drops of decinormal hydrochloric acid to 7 c.c. of venom solution interfered with the adsorption of venom by the charcoal in only one experiment and then only to a slight extent. Nineteen mice injected with the supernatant fluid of this mixture survived the injection, but two mice injected with quantities corresponding to $13\frac{1}{3}$ lethal doses, two injected with $6\frac{2}{3}$ and one injected with $3\frac{1}{3}$ lethal doses, died. These five mice had all been injected with the same solution, and their death was the only instance in which any of the mice injected with the supernatant fluid died. Of the eleven mice injected with the residue, only two died. The addition of weak acid to the venom-charcoal mixture may, therefore, cause the bond between the charcoal and the venom to be more easily broken after the injection of the residue into a living organism.

In other experiments we added first two drops of decinormal sodium hydroxide and subsequently the usual volume of charcoal to 7 c.c. of venom solution. Of 21 mice injected with the supernatant fluid, 1 died as soon as the

control, 3 lived longer than the controls, and 17 survived the injection. Several of the mice which survived the injection were quite markedly affected by the venom, showing weakness, as well as the typical changes in the eyes described in one of the preceding papers. These results suggest that alkalization of the venom solution interferes to some extent with the adsorption of the venom by charcoal. In experiments in which the charcoal residue of the alkaline suspensions was injected into mice, three survived the injection, whereas eight died a considerable time after the injection. Besides interfering with the adsorption of venom by charcoal, the addition of an alkali to the venom solution seems, therefore, to prevent the charcoal from firmly binding the adsorbed venom. Identical results were obtained with various venom solutions.

The addition of 2 c.c. of either dog or rabbit serum to 7 c.c. of venom solution interfered much more seriously with the adsorption of venom by the charcoal. All the animals injected with the supernatant fluid from the serum-charcoal-venom mixture died, 6 as soon as their controls and 12 after longer periods. Of the animals injected with the residue, 6 died and 4 survived. The toxic effect of the solid residue shows that although the addition of dog or rabbit serum to venom solution seems to prevent almost entirely the adsorption of venom by charcoal, still a small quantity must have been adsorbed and bound rather loosely by the charcoal.

Finally we tested the adsorption of venom by a mixture of equal parts of charcoal and lecithin (Kahlbaum). In this experiment a suspension of lecithin was obtained by rubbing and finely distributing 0.07 gram of lecithin in 7 c.c. of the venom solution. To this emulsion a quantity of charcoal equal to one-fourteenth of the bulk of the solution was added. Of the mice injected with the supernatant fluid five survived, and only one, which had been injected with $13\frac{1}{3}$ lethal doses of the venom, died. Of the three mice injected with the residue of the charcoal-lecithin mixture, only one died. This mixture of charcoal and lecithin acts, therefore, in approximately the same manner as the charcoal unaccompanied by the lecithin; the largest part of the venom is adsorbed and is held by the charcoal-lecithin mixture and in such a manner that it can not be absorbed by the injected animal. It is furthermore very probable that the charcoal rather than the lecithin held most of the venom; it was later found that the lecithin did not bind the venom as tightly as the charcoal.

ADSORPTION OF VENOM BY KAOLIN.

To the solution of dried venom a quantity of kaolin equal to one-fourteenth of the volume of the solution was added and the mixture kept in the shaking apparatus for 2 hours 30 minutes. Of 18 mice injected with this supernatant fluid 6 survived, 10 lived longer than their controls, and 2 died as soon as the controls. Of these last 2 mice 1 had received a quantity of fluid corresponding to $13\frac{1}{3}$, the other to $6\frac{2}{3}$ lethal doses. These experiments indicate that kaolin does not adsorb as much venom as charcoal, but it may, however, adsorb from 70 to 80 per cent of the venom.

Eight mice injected with the residue died. The kaolin seems to hold the adsorbed venom very loosely and the bond between the kaolin and the venom is easily broken after the injection of the kaolin into a living organism.

ADSORPTION OF VENOM BY ALUMINIUM OXIDE.

Two different samples of Al_2O_3 were tested. Three mice injected with the supernatant fluid obtained from the first sample (Eimer & Amend's preparation) died as soon as their controls. This solution was found to give an alkaline reaction.

A suspension of a second sample of Al_2O_3 gave a neutral reaction. In this experiment 12 mice injected wth the supernatant fluid survived while 1 receiving $3\frac{1}{3}$ lethal doses died. On the other hand, 7 mice injected with the residue of Al_2O_3 died; 6 of these had been injected with the residue of the second, 1 with the residue of the first (alkaline) sample of aluminium oxide. It appears, therefore, that aluminium oxide adsorbs the venom very well, but that here, as in the case of charcoal, the alkali interferes with the adsorption. The venom is, however, only loosely held by the aluminium oxide.

ADSORPTION OF VENOM BY OLIVE OIL.

By prolonged shaking an emulsion was made of one volume of olive oil in fourteen volumes of venom solution. All of the mice injected with this fluid after separation from the larger oil droplets died as soon as their respective controls. It was found that centrifugation alone did not free the fluid entirely of the finer oil droplets, and in order to obtain a fluid free from these droplets the mixture was passed through a Berkefeld filter and the filtrate injected into mice. All of the 6 mice injected with this filtrate ultimately died after having survived their controls for considerable periods of time. Furthermore, of 12 mice injected with the residue (containing both large and small oil droplets) only one died. It is, therefore, probable that some venom is adsorbed by the very fine oil droplets which are not separated from the fluid by the centrifugation, but that the amount of venom adsorbed is very small.

ADSORPTION OF VENOM BY LECITHIN.

In testing the adsorptive power of lecithin, three specimens were used, namely Merck's "Ovo," Kahlbaum's "Aus eigelb," and Agfa lecithin. In each case 0.07 gram of lecithin was mixed with 7 c.c. of venom solution. By rubbing the lecithin with small quantities of venom solution in a mortar a homogeneous, fine emulsion was obtained. Very fine particles of lecithin were held in suspension in the supernatant fluid, even after centrifuging. It was therefore necessary to filter the venom-lecithin mixture through a Berkefeld filter in order to free the supernatant fluid from particles of lecithin. Both the supernatant fluid obtained after centrifuging and the filtrate which had passed through the Berkefeld filter were tested.

Experiments with the supernatant fluid, decanted from the centrifuge tubes, showed that Merck's "Ovo" lecithin as a rule adsorbed somewhat less than 70 per cent of the venom; at times, however, it adsorbed somewhat more.

The Agfa lecithin adsorbed approximately the same proportion of the venom, while Kahlbaum's lecithin apparently adsorbed somewhat less than either of the two first-mentioned preparations. This was due to the fact that emulsions prepared with Kahlbaum's lecithin were finer and that consequently the supernatant fluid contained in this case a larger number of small particles of lecithin, to which venom adhered.

We find accordingly that the fluid obtained after filtration through a Berkefeld filter was not more toxic in the case of Kahlbaum's than in the case of Merck's lecithin; in most cases the lecithin adsorbed almost 70 per cent of the venom and in a few experiments even more.

All the mice injected with 2 c.c. of the washed and reemulsified residue of lecithin died, irrespective of the kind of lecithin used; one mouse injected with only 0.5 c.c. of the residue survived the injection. The amount of the emulsion of the lecithin residue injected in this last-mentioned case should have contained $3\frac{1}{3}$ lethal doses, provided all the venom had been adsorbed.

We may conclude from these experiments that lecithin adsorbs a large quantity of venom, but that the venom adsorbed exerts its toxic influence if injected with the lecithin to which it adhered.

The adsorptive power of a mixture of lecithin and cholesterin was also tested. 0.07 mg. each of lecithin (Kahlbaum) and cholesterin (Merck) were separately added to 7 c.c. of venom solution. Later a separation of the lecithin and cholesterin from this mixture was accomplished by means of a Berkefeld filter. Three mice, injected with the clear filtrate, survived, while three mice injected with the supernatant fluid obtained through centrifuging died as soon as their controls. One mouse injected with the lecithin-cholesterin residue died in a very short time. The addition of cholesterin to lecithin appears to increase the amount of venom adsorbed.

ADSORPTION OF VENOM BY THE ORGANS OF VARIOUS ANIMALS.

We investigated the adsorptive powers of the organs of the heloderma, turtle, frog, pigeon, guinea-pig, rabbit, and dog. Several organs were tested in each animal; in every case the kidney and liver and in most cases the brain also. In a few experiments some other parts were also used. As a rule, the organs were tested immediately after the death of the animal, before important autolytic processes could have taken place. The organs, cut into small pieces, were rinsed with salt solution, in order to wash out the blood, and then crushed in a mortar. 7 c.c. of venom solution were added to about 0.5 c.c. of organ pulp. This mixture was rubbed in the mortar until an even suspension of the organ pulp in the venom solution was obtained.

The further steps were similar to those described above. The solid particles of the various organs were separated from the solution in most cases by centrifugation and in a few cases by filtration through a Berkefeld filter.

Heloderma brain was tested with both fresh and dry venom. With the fresh venom all the animals injected with the supernatant fluid died: one mouse injected with a volume corresponding to four lethal doses, one injected

with a volume corresponding to one lethal dose, and two injected with volumes corresponding to two lethal doses lived a little longer than their controls; none, however, survived. Two of eight animals injected with supernatant fluid from the heloderma brain and dry venom mixture lived longer than their controls, but here also no animals survived. As might be expected from these results, the two mice injected with the heloderma brain-venom residue survived. The larger particles, which constitute the mass of the heloderma brain suspension, adsorb, therefore, very little venom, while to judge from the experiments with dog brain, to be reported later, a considerable part of the venom has been adsorbed by the small particles of brain contained in the supernatant fluid.

Heloderma liver adsorbed a small quantity of fresh venom; all five mice injected with the supernatant fluid from this mixture lived longer than their controls, and all but one lived 24 hours after the injection. With the dissolved dry venom solution the heloderma liver adsorbed as much as 85 per cent of the venom, but frequently less than 70 per cent. In some experiments mice injected with the smaller dose died, while in other experiments animals injected with a quantity equivalent to $6\frac{2}{3}$ lethal doses survived.

Two animals injected with the residue from the heloderma-liver venom mixture died, and two animals survived. On the whole, therefore, the liver of heloderma adsorbs a considerable part of the venom.

The adsorptive power of heloderma kidney, when mixed with fresh venom, was tested in only two animals, both of which died. They both, however, survived their controls for a considerable time; one had received a quantity of supernatant fluid corresponding to one lethal dose, the other a quantity corresponding to two lethal doses.

When heloderma kidney was mixed with a solution of dry venom, as much as 85 per cent of the venom was adsorbed in some cases, while in others less than 70 per cent. Four mice were injected with residue from the heloderma-kidney venom mixture; one of these died and three survived. In the cases when the residue was not toxic, the venom had possibly been held more firmly by the pulp, or it had been washed out with the salt solution used to wash the residue. The latter interpretation is, however, less probable. From all these experiments we may conclude that heloderma liver and kidney adsorb a considerable amount of venom.

One mouse injected with a mixture of venom solution and heloderma serum died approximately at the same time as its control.

Turtle brain, like heloderma brain, adsorbed apparently very little venom. Two mice injected, respectively, with one and two lethal doses of the supernatant fluid from the turtle-brain and venom mixture, lived only a little longer than their controls. The majority of the mice injected with the supernatant fluid from the mixture of turtle brain and dissolved dry venom died as soon as their controls. Two mice out of nine, however, survived the injection; one of these had been injected with a quantity of fluid corresponding to $6\frac{2}{3}$ lethal doses; but as in this experiment the mouse injected with $3\frac{1}{3}$

lethal doses lived only a little longer than the control, it would appear that some factor other than the adsorption of the venom was the cause for the survival of this animal. In another experiment one animal, injected with $3\frac{1}{3}$ lethal doses, survived. The residue of the turtle-brain venom mixture injected into mice caused death in one case, while the only other mouse injected survived. In the case of turtle brain we have to make the same reservation as in the case of heloderma brain, namely, that a considerable part of the venom may have been, and in all probability had been, adsorbed by the small particles suspended in the supernatant fluid. A small part had fixed itself upon the surface of the larger particles of the suspension.

Only two mice were injected with the supernatant fluid from the fresh-venom turtle-liver mixture and both of these died.

In experiments in which dissolved dry venom was used, turtle liver seemed to adsorb the venom better than turtle brain; 5 of 27 mice injected with the supernatant fluid survived. These five animals had for the most part been injected with quantities corresponding to $3\frac{1}{3}$ lethal doses of venom, in one case with a quantity equivalent to $6\frac{2}{3}$ lethal doses. Turtle liver may, therefore, adsorb as much as 85 per cent of the venom, and usually adsorbs more than 70 per cent. Three out of four injected with the residue of this turtle-liver venom mixture survived the injection. Whether venom was held very firmly or destroyed by the liver pulp or whether the venom was washed off by the salt solution we can not state, although it is improbable that the latter explanation is correct.

Two mice were injected with quantities of the supernatant fluid from the turtle-kidney fresh-venom mixture, equivalent to one and two lethal doses of venom respectively, and both of these animals survived; 8 of the 28 mice injected with the supernatant fluid from the mixture with dry venom survived, and 2 of the 8 had been injected with quantities corresponding to $6\frac{2}{3}$ lethal doses. The injection of the residue of turtle-kidney venom mixture was lethal in three out of four cases. Turtle kidney adsorbs approximately the same amount of venom as turtle liver.

Two mice were injected with the supernatant fluid from a mixture of venom solution and turtle egg; one of these, receiving $3\frac{1}{3}$ lethal doses, died as soon as the control mouse, while the other, injected with a quantity corresponding to $6\frac{2}{3}$ lethal doses, lived longer than the control. One mouse injected with venom mixed with turtle serum died as soon as its control. Neither the admixture of turtle egg or turtle serum diminishes the toxicity of the venom to any marked degree.

Of 10 mice injected with the supernatant fluid from a frog-liver venom mixture, 6 died as soon as their respective controls, 3 lived longer, and 1 survived the injection. The only mouse which survived had been injected with a quantity of fluid corresponding to $6\frac{2}{3}$ lethal doses. Hence it would seem that the adsorptive power of frog liver is very slight.

Of 3 mice injected with frog-liver venom residue, 1 died and 2 survived. These results agree with the findings in the experiments in which the supernatant fluid was injected.

The action of frog kidney was tested on 9 mice, only 1 of which (an animal injected with a large quantity of the supernatant fluid), died in as short a time as the control; 1 which had received a quantity corresponding to only $3\frac{1}{3}$ lethal doses of venom, survived, while 7 lived longer than their controls. Frog kidney adsorbs, therefore, a small quantity of venom, but less than heloderma or turtle kidney.

Frog-kidney venom residue was injected into 3 mice, none of which died. Since frog kidney adsorbs a small quantity of venom, frog kidney, like turtle liver and kidney and heloderma kidney, either holds the adsorbed venom sufficiently firmly to prevent the venom from being adsorbed very rapidly by the injected mouse or it destroys the adsorbed venom.

Three animals injected with the supernatant fluid from pigeon-brain venom mixture died as soon as their respective controls; one injected with the residue from the pigeon-brain venom mixture survived. Since the supernatant fluid from this mixture contained fine particles of brain matter, we can not exclude the possibility that pigeon brain adsorbs a certain amount of venom; it seems, however, that the quantity of venom adsorbed is small.

Of 9 mice injected with the supernatant fluid from pigeon-liver venom mixture, 2 injected with large quantities died as soon as their controls; 3 lived longer, while 4 survived the injection. Of the 4 surviving animals, 2 had been injected with quantities of venom corresponding to $3\frac{1}{3}$ lethal doses, 1 with a quantity corresponding to $6\frac{2}{3}$ lethal doses, and 1 with a quantity corresponding to $13\frac{1}{3}$ lethal doses. We may therefore conclude that pigeon liver adsorbs a certain amount, approximately from 70 per cent to 80 per cent of the venom. This conclusion is confirmed by an experiment in which 3 mice were injected with the residue from the pigeon-liver venom mixture; 2 of these died and 1 survived.

The supernatant fluid from a pigeon-kidney venom mixture was injected into 6 mice; 4 died as soon as the controls; 1, which had been injected with a quantity corresponding to $6\frac{2}{3}$ lethal doses, survived the injection. Pigeon kidney, therefore, seems to adsorb somewhat less venom than pigeon liver. Of the two animals injected with pigeon-kidney venom residue, one survived the injection.

Of 5 mice injected with the supernatant fluid from the mixture of fresh venom with the pulp of the whole brain of rabbits, 4 died as soon as the controls; the fifth lived a little longer than the control. Of 4 injected with the supernatant fluid from a mixture of venom with the gray matter of rabbit brain only, 2 lived longer than the controls, while the others died as soon as the controls.

In an experiment in which the white matter of rabbit's brain was used in place of the gray matter, 3 animals out of four lived longer than the controls. Here again a certain amount of venom had probably been adsorbed by the very small particles of the brain emulsion.

In 2 cases in which supernatant fluid from a fresh-venom rabbit-liver mixture was injected into mice, the animals died as soon as the controls. Of 3 mice injected, 2 with $13\frac{1}{3}$ lethal doses, and 1 with $6\frac{2}{3}$ lethal doses, respectively, from this rabbit-liver dry-venom mixture, died as soon as their controls, whereas

1 injected with a quantity corresponding to $3\frac{1}{3}$ lethal doses lived somewhat longer. Very little venom had, therefore, been adsorbed, and accordingly we find that a mouse injected with 2 c.c. of the residue of this rabbit-liver venom mixture survived.

Of 3 mice injected with the supernatant fluid from a mixture of fresh venom and rabbit kidney, 2 lived longer than the controls, and a third died as soon as the control. Little or no adsorptive effect of rabbit kidney was therefore evident. Similar results were obtained with the supernatant fluid when a solution of dry venom had been mixed with rabbit kidney, and, furthermore, one mouse injected with the residue from this mixture survived. Considerably less than 70 per cent of the venom is adsorbed by rabbit liver and kidney.

Nine mice injected with the supernatant fluid from a venom dog-brain mixture died as soon as their controls. Since even by prolonged centrifugation it was impossible to throw down some of the finest particles of the dog-brain venom emulsion,* we filtered this mixture through a Berkefeld filter in order to obtain fluid free from brain tissue; 2 animals injected with a quantity of the filtrate corresponding to $13\frac{1}{3}$ lethal doses and $6\frac{2}{3}$ lethal doses, respectively, lived longer than their controls, while 1 injected with a quantity corresponding to $3\frac{1}{3}$ lethal doses survived the injection. These results make it probable that the lethal effect of the non-filtered supernatant fluid was in part due to the very fine particles of brain tissue present in this fluid. These fine particles of brain-tissue which were removed by filtration had evidently adsorbed considerable quantities of venom and when injected into mice gave up the venom in sufficient quantities to cause the death of the animal. We may therefore conclude that, under the conditions of our experiments, dog brain adsorbs almost 70 per cent of the venom. We found that 2 of 3 mice injected with residue from dog-brain venom mixture survived; 1 died. It seems, therefore, very probable that the fine particles which remained in the supernatant fluid had adsorbed very large proportions of the venom; it still remains to be seen whether some venom had been removed from the brain-pulp during the process of washing.

Six mice were injected with the supernatant fluid from a venom dog-liver mixture; 5 died as soon as the controls; 1 injected with a quantity corresponding to $6\frac{2}{3}$ lethal doses survived. Six mice injected with the supernatant fluid from dog-kidney venom mixture all died as soon as their controls. It is therefore evident that dog kidney, like dog liver, adsorbed no venom, or almost none.

Two mice were injected with residue from the dog-liver venom mixture and 2 with residue from dog-kidney venom mixture; all survived. These results are in accord with the results obtained in the case of the supernatant fluid and confirm the conclusion that neither of these organs adsorbs appreciable quantities of venom.

Two series of six mice each, injected with the supernatant fluid from a mixture of venom with either washed dog-erythrocytes or the stroma of these cells, died as soon as their controls. Notwithstanding these results, injection of

*The supernatant fluid from the brain-venom mixtures contained fine particles of brain substance, not only in the case of dog, but of all other species as well; although more pronounced in the case of dog brain.

the residue from these mixtures proved that erythrocytes adsorb a certain amount of venom. One of the 2 mice injected with the residue from the dog-erythrocyte venom mixture died and the injection of the residue from the stroma-venom mixture caused the death of both of the mice injected. It is possible that a part of the erythrocyte-venom combination had dissolved in the supernatant fluid, and this dissolved substance may have caused the toxicity of the supernatant fluid. We may also have to consider the possibility that somehow dog erythrocytes increase the toxicity of venom. It is, however, quite clear that erythrocytes and stroma adsorb a certain amount of venom.

Of 9 mice injected with the supernatant fluid from a mixture of guinea-pig brain and dry venom, only that one injected with a quantity corresponding to $3\frac{1}{3}$ lethal doses survived; 2 injected with a quantity corresponding to $6\frac{2}{3}$ lethal doses lived longer than their controls, and the remaining 6 died as soon as the controls. Of five animals injected with the residue from the venom-brain mixture, 4 died. It is difficult to draw a definite conclusion from the results of these experiments, as the supernatant fluid contained particles of brain in suspension, which may have adsorbed venom, and their retention in the supernatant fluid may have increased its toxicity. It was thus necessary to test a fluid entirely free from these particles. We therefore passed the guinea-pig-brain venom mixture through a Berkefeld filter.

We then carried out a series of experiments comparing the supernatant fluid obtained by centrifugation, the Berkefeld filtrate, and also the Berkefeld filtrate of a guinea-pig-brain venom mixture, which, however, before the filtration, had not been shaken for the usual period of $2\frac{1}{2}$ hours, but which had been filtered immediately after mixing. Through this latter experiment we wished to determine whether the passage of the venom-brain through a Berkefeld filter caused either a diminution in the toxicity of the venom through adsorption of venom by the substance of the filter or through clogging of the pores of the filter by minute particles of the brain substance. We found the simple separation of the fluid from the solid portion of the venom-brain mixture by means of the Berkefeld filter did not cause any marked diminution in the toxicity of the filtrate; 4 mice injected with such a filtrate from a venom-guinea-pig-brain mixture died as soon as their controls, and 2 injected with quantities corresponding to $6\frac{2}{3}$ lethal doses lived a little longer than the controls.

All 5 mice injected with the filtrate from the guinea-pig-brain venom mixture (after it had been shaken for the usual period of time) lived longer than the controls, but none survived. In the control experiments in which the supernatant fluid from the guinea-pig-brain venom mixture was obtained by centrifuging, three animals, one injected with a quantity of fluid corresponding to $13\frac{1}{3}$ and two with a quantity corresponding to $6\frac{2}{3}$ lethal doses, lived longer than their controls, while three other animals died as soon as the controls. We may therefore conclude that guinea-pig brain adsorbs a certain amount of venom, but less than 70 per cent. Here, as in the case of dog erythrocytes and the brain substance of other species, we must consider the possibility of a toxic combination of venom and brain partly soluble in the supernatant fluid.

In the experiments in which the residue of guinea-pig-brain venom mixture was injected into mice, 4 of 5 died, an additional proof that guinea-pig brain adsorbs some venom.

In the experiments testing the adsorptive power of guinea-pig liver 26 animals were used; 3 injected with quantities of the supernatant fluid corresponding to $3\frac{1}{3}$ lethal doses of venom survived the injection, 12 lived longer than the controls, and 11 died as soon as the controls. Thus it is evident that guinea-pig liver may adsorb a considerable quantity of venom. That guinea-pig liver does adsorb venom is substantiated by the experiments in which the residue of the guinea-pig-liver venom mixture was injected into mice; 4 of 7 mice injected with this residue died.

Experiments were undertaken with guinea-pig liver similar to those carried out with guinea-pig brain, comparing the various methods of separation of the fluid from the solid portions of the mixtures. If the guinea-pig-liver venom mixture was filtered through a Berkefeld filter without having been placed in the shaker previously, the filtrate thus obtained was almost as toxic as the pure venom solution. Mice injection with the Berkefeld filtrate of the guinea-pig-liver venom mixture (shaken for $2\frac{1}{2}$ hours before the filtration), in the majority of cases did not die as soon as their controls (4 out of 6 lived longer than their controls), but none survived. Of 6 mice injected with supernatant fluid from the guinea-pig-liver venom mixture (separated by centrifugation) at the same time as these last-mentioned animals, 4 also lived longer than their controls; the remaining 2 injected with supernatant fluid died as soon as the controls.

In the experiments testing the adsorptive power of guinea-pig kidney, 5 of the 21 mice injected with the supernatant fluid, died as soon as the controls; 12 lived longer than the controls, and 4 which had received quantities of the supernatant fluid corresponding to $3\frac{1}{3}$ lethal doses recovered. We may, therefore, conclude that guinea-pig kidney adsorbs in most cases as much as 70 to 80 per cent of the venom. After injection of the residue from a guinea-pig-kidney venom mixture, 2 out of the 3 animals died. It is, therefore, evident that guinea-pig kidney, like guinea-pig liver, adsorbs venom, but that the venom is easily liberated from the organ pulp.

Three mice injected with a venom-egg albumen mixture died as soon as their controls. Egg albumen exerts, therefore, no antitoxic influence on venom.

CONCLUSIONS.

Of the various substances tested, charcoal adsorbs heloderma venom most completely. A quantity of charcoal equal to one-sixteenth of the volume of the venom solution adsorbs all or almost all of the venom, and the adsorbed venom is held firmly by the charcoal when injected into a living organism.

Carmine adsorbs venom completely or almost completely, when present in a quantity equal to one-quarter of the volume of the venom solution, but does not adsorb very much more than half of the venom when present in a quantity equal to one-eighth of the volume of the venom solution.

Aluminium oxide, when present in a quantity equal to one-sixteenth of the

volume of the venom solution, adsorbs most of the venom, probably as much or only a little less than charcoal. Unlike charcoal, the aluminium oxide, when injected into a living organism, gives off the venom.

Kaolin does not adsorb venom as well as the three first-mentioned substances, and probably adsorbs but little more than 70 per cent of the venom. Like aluminium, kaolin holds the venom but loosely.

Lecithin adsorbs considerable quantities of venom, but much less than charcoal; its action appears to vary considerably; the addition of cholesterin to the lecithin does not markedly alter the adsorption of venom. The venom adsorbed by lecithin readily exerts its toxic action after subcutaneous injection of the residue, due to the rapid absorption of the injected lecithin and the subsequent dissociation of the venom-lecithin combination.

The addition of dog or rabbit serum to the venom solution interferes very markedly with the adsorption of venom by charcoal; the addition of a small amount of alkali to the venom solution interferes considerably less than the addition of serum. Under the influence of alkali the venom-charcoal combination is very much more easily dissociated in the body, alkali preventing the strong fixation of the venom to the charcoal.

In a similar manner an alkaline aluminium-oxide preparation adsorbs the venom much less strongly than a neutral preparation.

The addition of a small quantity of a weak acid to the venom solution interferes only slightly with the adsorption of venom by charcoal.

Olive oil adsorbs but little venom, and a considerable part of that adsorbed is held by the very finest particles, as in the case of the venom adsorbed by lecithin.

If we turn to the adsorption of heloderma venom by suspensions of organs, we can state in general that the pulp of organs adsorbs less venom than charcoal, aluminium oxide, or carmine. The behavior of brain is very similar to emulsions of lecithin. In both cases the small particles that remain in the fluid after centrifugation adsorb a certain quantity of venom. Even after filtration through a Berkefeld filter these particles are not removed thoroughly and the supernatant fluid retains a certain degree of toxicity. The whole brain adsorbs less venom than emulsions of lecithin. That a certain amount of venom is adsorbed by brain pulp is shown by the fact that the unfiltered is more toxic than the filtered fluid, and, furthermore, by the toxicity of the residue which we observed in certain cases. The fact that even after filtration the filtrate obtained has not become harmless and that in a number of cases the mice injected with the residue survived, shows that much of the venom remained unadsorbed.

The brain of various species does not on the whole adsorb any more venom than liver and kidney. This is clear in the case of guinea-pig. In no case did the brain of any of the species tested adsorb as much venom as the liver and kidney of heloderma and turtle. In this respect heloderma venom seems to differ from cobra venom, which, according to Flexner and Noguchi, lost much more of its toxicity after adsorption by brain than by other organs. Also, Calmette and Rogers found that brain adsorbs much snake venom.

On the whole, kidney and liver act similarly in the case of the various species; when a definite adsorbing power is present in the kidney of a certain species, the liver will behave in approximately the same manner.

We find in certain cases that the residue is not particularly toxic, although much venom had been removed from the supernatant fluid through adsorption. In such cases (heloderma and turtle liver and kidney) several possible explanations have to be taken into consideration. The venom may be so strongly attached to the residue that at a given time very little venom enters into the circulation—not more than can easily be eliminated or neutralized by the body; or the venom may in part have been rendered innocuous through chemical action of certain parts of the organ pulp on the venom; or—a not very probable assumption—a part of the venom may have been removed from the residue in the process of washing. Further investigations will have to decide between these various explanations.

In the case of dog erythrocytes we found both the supernatant fluid and the residue toxic. This apparently contradictory behavior will also have to be investigated in further experiments.

In a preliminary manner we may arrange the organs (liver and kidney) of various species of animals in the order of their decreasing adsorptive power for heloderma venom: *Heloderma*, turtle, pigeon, guinea-pig, frog, rabbit, and dog. This order suggests a connection between the natural relationship of various species and their adsorptive power for heloderma venom. *Heloderma* and turtle, both reptiles, showed in our experiments the greatest adsorptive power. The natural immunity of *Heloderma* against its own venom may possibly in part depend on a specific adsorbing and neutralizing power of certain of its organs. These results should, however, at present not be regarded as definite, the number of our experiments being as yet relatively small; but they should serve as indicating the direction of further experiments, which might yield definite results of importance.

We may summarize some of our results as follows: In the case of heloderma venom no specific adsorptive or neutralizing action of the brain exists; other organs, like liver and kidney, adsorb in the case of some animals equally as much, and in other cases even more venom than the brain.

The adsorption of venom by organ pulp is considerably inferior to the adsorption by certain other materials, like charcoal, carmine, or aluminium oxide.

There is some indication that differences exist in the adsorptive power of the organs of different species of animals, and that the organs of heloderma and of animals nearly related to *Heloderma* adsorb more venom than some other animals less nearly related, and that the natural immunity of *Heloderma* to its own venom may in part depend on this specific adsorptive power. The number of our experiments is perhaps not yet large enough to establish these conclusions; they, however, suggest such an interpretation. It remains for further experiments to decide definitely the value of this hypothesis.*

*Wolff-Eisner also suggested that the liver and perhaps other organs were of importance in protecting the brain from the action of certain poisons and in conferring immunity against certain toxic substances. (Centralblatt f. Bact., Originale, Bd. 47, 1908; Bd. 48, 1908-09.

Experiments with fresh venom.

Material.	Died as soon as controls.						Lived longer than controls.						Survived.					
	10	7	6	5	2	1	10	7	6	5	2	1	10	7	6	5	2	1
Charcoal, ½ bulk	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	1	3	1
Charcoal, ¼ bulk	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1	..
Charcoal, ⅛ bulk	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	..	2	..
Charcoal, $\frac{1}{10}$ bulk	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1	..
Charcoal, $\frac{1}{32}$ bulk	..	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..
Charcoal, ⅛ bulk (mixed only 15 minutes)*	..	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..
Carmine, ½ bulk	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	1	1	..
Carmine, ¼ bulk	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1	..
Carmine, ⅛ bulk	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1	..

*The controls, charcoal ⅛ bulk mixed 2½ hours, also died as soon as controls.

Experiments with dissolved dry venom.

Material.	Died as soon as controls.			Lived longer than controls.			Survived.			Residue, 2 c.c.	
	13⅓	6⅔	3⅓	13⅓	6⅔	3⅓	13⅓	6⅔	3⅓	Survived.	Died.
Charcoal	..	..	..	..	1	..	6	4	5	8	..
Charcoal and lecithin	..	..	..	1	..	..	1	2	2	2	1
Charcoal and acid	..	..	..	2	2	1	7	6	6	9	2
Charcoal and alkali	1	..	..	..	1	2	6	6	5	3	8
Charcoal and dog serum	..	..	..	1	1	1	..	..	..	..	1
Charcoal and rabbit serum	1	2	3	4	3	2	..	..	..	4	5
Kaolin	1	1	..	2	4	4	3	1	2	..	8
Al_2O_3, alkaline	1	1	1	..	..	..	..	..	..	..	1
Al_2O_3, neutral	..	..	..	..	..	1	4	4	3	..	6
Olive oil, centrifuged	6	6	6	..	..	..	..	..	..	11	1
Olive oil, Berkefeld	..	..	..	2	2	2	..	..	..	..	..
Lecithin (Merck)	2	..	1	..	1	..	1	2	2	1	3
Lecithin (Merck) Berkefeld	..	..	..	1	..	..	..	..	1	..	..
Lecithin (Kahlbaum)	4	4	4	1	1	..	..	1	1	1	5
Lecithin (Kahlbaum), Berkefeld	..	..	..	2	..	3	2	1	1	..	..
Lecithin, agfa	..	1	..	..	..	..	..	..	1	..	2
Lecithin and cholesterin	1	1	1	..	..	..	..	..	..	..	1
Lecithin and cholesterin, Berkefeld	..	..	..	..	..	..	1	1	1	..	..

Experiments with fresh venom.

Material.	Died as soon as controls.						Lived longer than controls.						Survived.					
	7	5	4	3	2	1	7	5	4	3	2	1	7	5	4	3	2	1
Heloderma:																		
Brain	1	..	..	..	1	1	..	..	1	..	2	1	..	..	..	..	..	..
Liver	..	..	..	..	..	..	1	..	1	..	2	1	..	..	..	..	..	..
Kidney	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..
Testes	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..
Turtle:																		
Brain	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..
Liver	..	..	..	..	..	1	..	..	..	..	1	..	..	..	..	..	..	..
Kidney	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1
Rabbit:																		
Brain	1	1	1	1	1	..	..	..	..	..	..	1	..	..	..	..	..	..
Brain, gray	..	..	..	..	1	1	..	..	..	..	1	1	..	..	..	..	..	..
Brain, white	..	..	..	..	1	..	..	..	..	..	1	2	..	..	..	..	..	..
Kidney	..	..	..	..	1	..	..	..	..	..	1	1	..	..	..	..	..	..
Liver	..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..

Experiments with dissolved dry venom.

Material.	Died as soon as controls.			Lived longer than controls.			Survived.			Residue, 2 c.c.	
	13⅓	6⅔	3⅓	13⅓	6⅔	3⅓	13⅓	6⅔	3⅓	Survived.	Died.
Heloderma:											
Brain	3	2	1	..	..	2	..	..	..	2	..
Liver	1	1	1	6	4	2	2	3	6	2	2
Kidney	2	3	2	7	3	2	..	2	5	3	1
Plasma	1	..	..	..	..	..	..	..	..	..	..
Turtle.											
Brain	3	..	2	..	1	1	..	1	1	1	1
Liver	2	1	4	7	6	2	..	1	4	3	1
Kidney	3	2	1	6	4	4	1	2	5	3	1
Egg	..	..	1	..	..	..	..	..	1	..	..
Plasma	1	..	..	..	..	..	..	..	..	..	..
Frog:											
Liver	4	..	2	..	2	1	..	1	..	2	*1
Kidney	1	..	..	2	3	2	..	..	1	3	..
Pigeon:											
Brain	1	1	1	..	..	..	..	..	..	1	..
Liver	2	2	..	..	..	1	1	1	2	1	2
Kidney	2	1	1	..	..	1	..	1	..	1	1
Rabbit:											
Liver	1	1	..	..	..	1	..	..	..	1	..
Kidney	1	1	..	..	..	1	..	..	..	1	..
Guinea-pig brain, not put in shaker, but filtered through Berkefeld	2	..	2	..	2	..	..	..	..	..	..
Guinea-pig brain, mixed and centrifuged	3	1	2	..	2	..	..	..	1	1	4
Guinea-pig brain, Berkefeld filter	..	..	..	2	1	22	..	..	..	..	..
Guinea-pig liver not put in shaker, but filtered through Berkefeld	1	1	2	1	1	..	..	..	..	..	..
Guinea-pig liver, mixed aad centrifuged	4	2	5	5	6	1	..	..	3	3	4
Guinea-pig liver, Berkefeld filter	1	..	1	1	2	1	..	..	..	..	..
Guinea-pig kidaey	2	1	2	5	4	3	..	..	4	1	2
Dog brain centrifuged	3	3	3	..	..	..	..	..	..	2	1
Dog brain, Berkefeld	..	..	..	1	1	1	..	..	1	..	..
Dog liver	2	2	1	..	..	..	..	..	1	2	..
Dog kidney	2	2	2	..	..	..	..	..	..	2	..
Dog erythrocytes	2	2	2	..	..	..	..	..	..	1	1
Dog stroma	2	2	2	..	..	..	..	..	..	..	2
Egg albumen	1	1	1	..	..	..	..	..	..	..	..

*Mixed with bile.

XV.

BIOCHEMICAL STUDIES UPON THE VENOM OF HELODERMA SUSPECTUM.

By Carl L. Alsberg.

BIOCHEMICAL STUDIES UPON THE VENOM OF HELODERMA SUSPECTUM.

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The most important study of the chemical nature of the venom of *Heloderma* was made by Santesson.* His conclusions were in the main that no alkaloidal substances were present, but that the toxic principle was part nuclein and part albumose. He also discovered that boiling a solution of the venom, even in the presence of acetic acid, did not materially injure its toxicity; and that the toxic principle was precipitated but not coagulated by alcohol and similar agents. The purpose of the present investigation was to endeavor to isolate the toxic principle or, failing that, to extend the observations of Santesson. It was expected that these questions might be solved because more material was available than Santesson had, and because in recent years, through the work of Faust on cobra† and rattlesnake‡ venom, our knowledge of the chemistry of venoms of reptiles has been greatly extended. It was hoped that it might be possible to isolate the active principle of heloderma venom by means devised by Faust.

The first step in this investigation was to apply to the dried venom the various methods used by Faust. To test the toxicity in the course of this chemical work mice were invariably used and the solutions injected subcutaneously.

Faust's copper method was first tried. 0.2 gm. of finely powdered venom was suspended in 15 c.c. of water and allowed to extract for an hour. A small portion remained undissolved. It was removed by filtration and thoroughly washed with water on the filter. It was dissolved on the filter in 2 c.c. of 0.8 per cent sodium-chloride solution rendered weakly alkaline with sodium carbonate. 0.5 c.c. was injected into a white mouse of 20 gm. beneath the skin of the abdomen. The following morning the mouse was found dead, lying flat and relaxed on its abdomen, as though it had died of progressive paralysis without convulsions. The experiment was repeated with the remainder of the sodium-carbonate solution neutralized with a trace of hydrochloric acid. Neutralization caused the solution to become opalescent; a slight excess of acid caused a precipitate. The result of the injection, as was to be anticipated, was entirely the same as with the unneutralized solution, for the amount of sodium carbonate used was very slight, quite insufficient to produce serious effects by itself, as control injections of pure sodium-carbonate solutions of the same strength showed.

*Santesson, C. G. Ueber das Gift von *Heloderma suspectum* Cope, einer giftigen Eidechse, Nordiskt Medicinisk Archiv., Festband Axel Key, 1897, Nr. 5.

†E. St. Faust. Ueber das Orphiotoxin aus dem Gifte der Ostindischen Brillenschlange (*Naja tripudians*), Archiv für experimentelle Pathologie und Pharmakologie, Bd. 56, S. 236 (1907).

‡E. St. Faust. Ueber das Crotalotoxin aus dem Gifte der Nord-Amerikanischen Klapperschlange (*Crotalus adamanteus*), *ibid.*, Bd. 64, S. 214 (1911).

The part of the venom insoluble in water could not be freed completely from toxic material without very long-continued washing, though it is likely that the toxic material is merely adsorbed or otherwise mechanically held in the insoluble portion. The aqueous solution, which was slightly yellow and faintly opalescent, was treated with a little chemically pure neutral copper acetate and then with weak potassium hydrate, drop by drop, till the reaction became distinctly alkaline and no further precipitate formed. The liquid and the precipitate were then separated by centrifugation. The precipitate was dissolved in water acidulated with acetic acid and then carefully made alkaline again. The precipitate was again separated by centrifugation and washed repeatedly. It was finally freed from copper by first washing with 95 per cent alcohol; then with alcohol containing a little hydrochloric acid, till all the copper was removed. There remained only an insignificant precipitate, which was washed free from chlorine with alcohol.

Only a few milligrams of material remained. The adherent alcohol was allowed to evaporate spontaneously and the residue was dissolved in 0.75 c.c. of normal saline solution. Of this 0.5 c.c. were injected into a mouse of 22 gm. weight beneath the skin of the abdomen. The mouse was uncomfortable for several hours, but within 24 hours was apparently quite normal again. Hence either the treatment destroyed the toxic substance or else the copper precipitate did not carry it down. The venom of *Heloderma* must therefore be different from that of the cobra and the rattlesnake. According to Faust's observations, the toxic principles of the latter are precipitated with copper hydrate.

Further observations showed that in all probability no appreciable quantities of the venom were destroyed by the copper treatment. The clear solution decanted from the precipitate of copper hydrate showed a strong typical biuret reaction. It was carefully acidified with acetic acid. An abundant white precipitate formed, which was separated by centrifugation from the liquid. To remove any traces of copper it may have contained it was carefully washed with a little water containing a trace of acetic acid; then dissolved with the aid of sodium carbonate and again precipitated with acetic acid and washed. It was finally washed with an abundance of weak alcohol. The precipitate, when finally quite free from copper, was dissolved in 10 c.c. of normal saline solution with the help of very little very weak sodium carbonate. 0.25 c.c. was injected into a series of white mice of 20 to 25 gm. weight; all died with characteristic symptoms. The protocol of one experiment may serve as an example:

White mouse weighing 20 gm. received 0.25 c.c. beneath the skin of the abdomen at $4^h\ 23^m$ p. m.; became uncomfortable and sick within a few minutes; at $4^h\ 41^m$ p. m. it fell over on one side for a moment and then had convulsions, then recovered somewhat. This was followed by dyspnea and paralysis, the mouse lying on one side. Finally it was quite paralyzed, dying at $5^h\ 15^m$ p. m.

It is therefore evident that the copper method of Faust is not applicable to heloderma venom, since the copper precipitate carries down little, if any, of the toxic principle.

Thereupon the metaphosphoric acid method was tried. The aqueous venom solution prepared as in the previous experiment was saturated with sodium chloride, weakly acidulated with acetic acid, and the vessel containing the solution immersed for 15 minutes in a water-bath heated to 90 to 95° C. It was then quickly chilled by immersion in ice-water and filtered. The precipitate was washed on the filter with distilled water till the greater part of the chlorine was removed. It was then dissolved in 10 c.c. distilled water with the help of a little sodium carbonate and the alkalinity partially neutralized with hydrochloric acid. Complete neutralization was not feasible because of the formation of a precipitate. 0.5 c.c. of the faintly alkaline solution, which contained some sodium chloride, was injected into a mouse of 20 gm. beneath the skin of the abdomen at 3 p. m. The mouse was uncomfortable and restless at first. at $3^h 45^m$ it became quiet and plainly quite sick; at $3^h 55^m$ it had a convulsion, and thereafter it became evident that there was the beginning of paralysis with dyspnea, paralysis being most pronounced in the hind leg; at 6 p. m. the paralysis was far advanced, though the mouse still reacted to pinching; it died between 6 and 8 p. m.

The filtrate obtained from the above precipitate was dialyzed till all the sodium chloride was removed. Through the addition of wash-water and through the dialysis the original volume was much increased. It was therefore concentrated at a pressure of 2 mm. of mercury, barium oxide being used as drying agent. Within 12 hours it was thus reduced to about the original volume of 15 c.c. In 1 c.c. of this solution, which gave a strong biuret reaction, 8 mg. of sodium chloride were dissolved to make it approximately isotonic with a normal saline solution, and 0.5 c.c. injected into a mouse of 18 gm. The injection was made at $10^h 30^m$ a. m.; at $10^h 50^m$ the mouse was somewhat affected but still restless; at $11^h 30^m$ a. m. paralytic effects began to be noted; at 12 noon the effects were marked; at $12^h 15^m$ the mouse was lying on its side; at $12^h 45^m$ it was dead. Evidently heating and dialysis had not destroyed the activity, though it would appear that the solution had lost somewhat in strength. This is undoubtedly due, in part, to the occlusion in the precipitate obtained with acetic acid, sodium chloride, and heat of some of the toxic principle. It is probably also due in some measure to the concentration of the solution and to the dialysis. Other experiments have shown that concentrating solutions of the venom, freed from the greater part of their protein, causes them to lose toxicity; and that dialysis also causes some loss of activity. As the investigations of Cooke and Loeb have shown, the toxic principle is able to dialyze slowly through membranes.*

The remaining dialyzed solution was then treated with small quantities of freshly prepared 10 per cent metaphosphoric acid solution, avoiding an excess; a slight, very fine, flocculent precipitate formed, which could be removed completely only with the greatest difficulty. The ordinary, fine, ashless filter paper used in quantitative analysis was quite ineffective. A clear, non-opalescent solution was finally obtained by filtering many times through the same small,

*Cf. the paper by Elizabeth Cooke and Leo Loeb.

hardened filter paper. A portion of the filtrate thus obtained was carefully neutralized with sodium carbonate. The solution was almost colorless and gave no trace of the biuret reaction. Of the neutralized solution 0.5 c.c. was injected into a mouse of 20 gm. beneath the skin of the abdomen. The mouse became restless; after half an hour, some dyspnea appeared with possibly some weakness; but these passed away, so that on the following day the mouse was quite well. As a dose of 0.5 c.c. produced so few effects, 3 hours later 1.6 c.c. of the same solution were injected into a fresh mouse of 22 gm. beneath the skin of the abdomen. Such a relatively large amount of liquid always causes a great deal of discomfort, but for some hours little else was noticeable. The injection was made at 3 p. m.; at 5 p. m. some paralysis and dyspnea began to appear; at 6 p. m. the mouse was weak and lay flat upon its abdomen as though paralyzed, but it still reacted to pinching; at $6^h\ 46^m$ p. m. it no longer reacted to pinching, but was still able to move about feebly. It was not again observed till the following morning, when it had apparently recovered. By the following evening it was quite well and vigorous.

It was thought that perhaps the failure of this method might be due to the quality of the metaphosphoric acid used; therefore the entire experiment was repeated with metaphosphoric acid prepared from phosphorus pentoxide, as recommended by Abel and Ford.* This modification did not alter the result. Evidently this method, which has been so successful in the hands of Faust and of Abel and Ford, is not applicable to heloderma venom, because so much of the toxic material is held in the precipitate produced by acetic acid and heat, while almost all the remainder is carried down with the metaphosphoric acid precipitate.

The next method tried was the precipitation of the protein with dialyzed colloidal iron. A solution of 0.2 gm. venom in 15 c.c. of water was prepared as before. On the addition of the colloidal iron a very heavy and abundant precipitate was formed. The addition of iron was continued until a precipitate no longer resulted. The precipitate was removed by centrifugation. The clear supernatant liquid did not give the biuret reaction and contained but a very small amount of material in solution. 0.5 c.c. was injected into a mouse of 19 gm. beneath the skin of the abdomen at 11 a. m. All day the mouse showed no symptoms except those due to the injection, and on the following morning was quite well. A second mouse of 22 gm. received a similar injection of 1.5 c.c. at 2 p. m. This mouse showed moderate paralytic symptoms from 5 to 8 p. m.; but on the following morning it had quite recovered. Evidently the toxic material is carried down with the protein by the colloidal iron.

The toxic principle seems to differ from that of the cobra and of the rattlesnake in the greater ease with which it is adsorbed by all kinds of precipitates. Thus a solution of 0.05 gm. venom in 5 c.c. of water was filtered from the insoluble portion and freed from coagulable protein. The solution obtained was very toxic. It was then shaken with a little pure kaolin. The kaolin was

*Abel, J. J., and Ford, W. W. On the poisons of *Amanita phalloides*, Journal of Biological Chemistry, vol. II, p. 278 (1907).

removed by filtration. The filtrate was practically devoid of toxicity. The kaolin on the filter paper was then extracted on the filter with a little very weak acetic acid. The extract gave a distinct biuret reaction and was quite toxic, though not as powerful as the original solution.

Since the only precipitate which did not carry down the toxic principle was the copper precipitate; and since this was formed in alkaline solution, it seemed possible that a method by which protein is removed from an alkaline solution might not carry down the toxic principle. Such a method is the uranyl acetate method used by Kowalewsky,* Jacoby†, Glaessner,‡ Abel and Ford,§ and others. The procedure is to render the solution faintly alkaline with sodium carbonate and then to precipitate the protein with a saturated solution of uranyl acetate. A solution of venom, prepared as in the first experiment, was treated in this way. It was rendered weakly alkaline with sodium carbonate, and uranyl acetate was added till all the biuret-giving material had been precipitated. The precipitate was then removed by filtration. The filtrate was dialyzed till free from uranium and then concentrated *in vacuo* to the original volume; 1 c.c. injected into a mouse did not produce any characteristic symptoms. The precipitate produced by the uranyl acetate was then dissolved with the aid of acetic acid and dialyzed till free from uranium. It was then concentrated to a volume of 15 c.c. 0.25 c.c. was injected into a mouse of 18 gm. The symptoms set in very rapidly and death ensued within an hour. It is therefore evident that uranyl acetate is not a suitable reagent for purifying the venom.

Thereupon 0.2 gm. of venom finely powdered was treated for a few hours with 10 c.c. glacial acetic acid. Only a portion was dissolved. The residue was removed by filtration. It was gelatinous and semi-translucent. Most of it dissolved readily in water. The solution was filtered, the filtrate being quite opalescent. The filtrate was precipitated with twice its volume of alcohol. A relatively scanty precipitate formed. This was separated by filtration and thoroughly washed with alcohol. It was then dissolved in 1.5 c.c. of normal saline solution, neutralized with sodium carbonate and 0.5 c.c. injected into a mouse of 26 gm. beneath the skin of the abdomen. The injection was made at 4 p. m. The animal showed no special symptoms that afternoon, but was very quiet; the next morning it was found dead, lying flat on its abdomen, much in the position in which it had been last observed on the preceding afternoon. Evidently while the glacial acetic acid extracted most of the toxic principle, some of it remained undissolved.

The glacial acetic-acid solution of the venom was treated with twice its volume of 95 per cent alcohol. The flocculent precipitate formed was allowed to settle over night and then removed by filtration. After washing thoroughly with weak alcohol (alcohol 95 per cent 2 parts, water 1 part) it was dissolved in 1.75 c.c. of normal saline solution. It was slightly acid and gave a power-

* Zeitschr. f. anal. Chem., Bd. 24, S. 551 (1895).
† Zeitschr. f. physiol. Chem., Bd. 30, S. 135 (1900).
‡ Beiträge (Hofmeister) z. Chem. Physiol. u. Path., Bd. I., S. 1.
§ Journ. Biological Chemistry, vol. VII, p. 273 (1907).

ful biuret reaction. After neutralizing with sodium carbonate 0.75 c.c. was injected into a mouse of 30 gm. at $4^h\ 10^m$ p. m. The effect was noticeable almost at once. Besides the usual symptoms there seemed to be a good deal of pain and irritation at the site of injection. At $5^h\ 10^m$ p. m. the hind legs were completely paralyzed. At $5^h\ 40^m$ p. m. respiration was feeble, though the animal still struggled occasionally. At $6^h\ 30^m$ p. m. the respiration was still more feeble. The animal was not observed during the night, but was found dead the following morning in the position in which it had last been seen the evening before.

The alcoholic filtrate was treated with an equal volume of 95 per cent alcohol. A second precipitate formed, apparently, about half as voluminous as the first. This was separated from the alcohol and dissolved in 1 c.c. of normal saline solution. It gave a rather weak biuret reaction. After neutralization with sodium carbonate 0.5 c.c. was injected into a mouse of 21 gm. Symptoms set in almost at once and ran a rapid course, the mouse dying within an hour. Paralysis set in so soon there were almost no convulsive symptoms.

The alcoholic filtrate was then treated with an equal volume of ether. A slight precipitate formed, which was allowed to settle over night. It was then removed by filtration and dissolved on the filter in 10 c.c. of normal saline solution. While the previous fractions obtained by precipitation with alcohol did not dissolve readily in water, this fraction was very soluble, yielding a perfectly clear, colorless, and faintly acid solution. This was concentrated *in vacuo* for 14 hours to a volume of 1.5 c.c.; of this, 0.25 c.c. was injected into a mouse of 23 gm. The mouse was affected almost at once; there was no struggle, but a rapid progressive paralysis, the animal dying in 85 minutes. This experiment was repeated upon a second mouse with quite similar results. The remainder of the solution was examined with great care for biuret-giving substances, but the reaction was quite negative.

The alcohol-ether filtrate was concentrated by an air-current until all the ether had been removed. Water was added to the alcoholic solution remaining. An appreciable amount of precipitate was formed, soluble in ether and capable of being extracted from its suspension in weak alcohol by ether. It was removed in this fashion: the ether evaporated, and the residue suspended in normal saline solution. When injected into a mouse it produced no untoward effects. There seems to be present in the venom an appreciable amount of fatty, or at least of ether-soluble, material. Whenever a solution of the venom was precipitated with alcohol an appreciable amount of material remained dissolved.

The entire experiment with glacial acetic acid was twice repeated with somewhat larger quantities of venom (0.5 gm.). In each instance the results were essentially the same. After all the material that could be precipitated from the glacial acetic-acid solution with alcohol had been removed a further slight precipitate could be obtained by the addition of ether. This precipitate was always very small in amount—not more than a few milligrams. In one of these experiments it did not give the biuret reaction; in the other it did.

The quantities which had to be used for this reaction were unfortunately of necessity so small that this does not finally prove the toxic principle to be non-protein, though it renders it exceedingly probable. The material was exceedingly toxic, though the quantity available was insufficient for quantitative determinations of toxicity. The impression obtained, however, was that it was very much more toxic than any of the other preparations.

The efforts to purify the venom by this method were not pursued further, because of the insufficient yields. Since the toxic principle withstood the action of glacial acetic acid it was thought that it might perhaps be purified by means of peptic digestion. Therefore 0.1 gram was dissolved in 10 c.c. of 0.15 per cent hydrochloric acid. It was filtered and 5 mg. of pepsin added. It was placed in the thermostat at 38° C. for 19 hours. During this time a few fine floccules had separated (nuclein?). These were removed by filtration; 0.3 c.c. of filtrate was rendered neutral and injected into a mouse of 18 gm. without producing much effect in 2 hours; but on the following morning the animal was dead. The material was replaced in the thermostat for 5 days. Then 0.3 c.c. was neutralized and injected into a mouse of 20 gm.; it did not become very sick and recovered. The experiment was repeated with the same result.

The experiment was repeated with tryptic digestion. After 18 hours of digestion the injection of 0.25 gm. into a mouse of 28 gm. produced a severe intoxication with paralysis, but ultimate recovery.

It is therefore evident that while the venom withstands peptic and tryptic digestion for a considerable time, the resistance is not great enough to warrant the use of digestion in the process of purification.

The best results, after many trials, were finally obtained by a modification of one of the methods used by Faust in his recent paper on the venom of the rattlesnake.* The filtered venom solution was treated with acetic acid as long as a precipitate formed. This precipitate was removed by centrifugation and repeatedly washed. There is at this stage a considerable loss of activity, for, in spite of thorough washing, this precipitate, which is either mucine or nuclein, retains a high degree of activity. This phenomenon was also observed by Santesson and led him to conclude that the active agent was in part nuclein. The filtrate, which was active, was treated very carefully with weak sodium carbonate till only very weakly acid. It was then rapidly heated to boiling over a free flame to coagulate the small amount of coagulable protein. It is very important that the acidity be right. If it is not, clear filtration is exceedingly difficult and tedious. As soon as the solution had come to a boil it was rapidly chilled with ice-water and the very slight coagulum removed by filtration. The clear filtrate was then dialyzed till free from chlorine. Dialysis should not be continued beyond this point, for there is a distinct loss of activity in the process. The dialyzed solution was then concentrated over sulphuric acid *in vacuo*, saturated with ether, and transferred to a centrifuge tube. It was then put in a centrifuge placed in the cold-storage room at a temperature of −18° F. The centrifuge was set in motion. From time to time it was stopped

*Op. cit.

and the tube examined. Gradually three layers were formed—an upper layer of fine ice-crystals; a middle layer rather cloudy or even milky from the separation of material due to the chilling; and a sediment consisting of fine precipitate formed by the chilling. This process was allowed to go on until the layer of ice-crystals had filled about half the tube. Then the tube was removed, a rift made with a file at the lower limit of the ice layer, and the tube held over a dish and cracked with a hot wire at the level of the file-scratch. The part of the tube containing the ice was thus removed. As much of the liquid above the sediment as possible was removed and the sediment rinsed into a fresh centrifuge tube with as little water as possible. It was warmed till the solution became clear and the freezing and centrifuging repeated. After the fourth freezing a solution was obtained which was exceedingly active, yet gave not the slightest trace of biuret reaction. The amount of material in it was very slight, for when working with small quantities of material, as was unfortunately necessary, the losses are relatively considerable, due to the imperfect separation of the active principle by freezing. By using larger quantities these losses would undoubtedly be lessened. The precipitate obtained in the first freezing was never free from biuret-giving material. Faust reports that in his experiments this was sometimes the case. The solutions which he subjected to cold seem to have contained less protein than the heloderma solution similarly treated, because colloidal iron removes protein better than coagulation. The heloderma solutions still contained much biuret-giving material and a part of this is precipitated on freezing. On the second freezing, the dilution of the biuret material being greatly reduced, less or none is frozen out. Finally, the yields seem to be less with this method for heloderma venom than for rattlesnake venom. A good deal of the active principle is adsorbed in the acetic-acid precipitate; not all is precipitated in the chilling process; the clear liquid was always quite toxic. The solution finally obtained contained very little material in solution. The doses injected could not have contained more than a small fraction of a milligram of the toxic principle. The animals were affected almost immediately. There was usually a short convulsion followed by rapid progressive paralysis and death in from 14 to 60 minutes.

Though many of the experiments performed in the effort to isolate the active principle yielded negative results so far as the purification of the toxine is concerned, it was still thought advisable to describe the typical experiments in some detail, because they furnish information concerning the behavior of the venom to various reagents. While this investigation, unfortunately, has not accomplished all that was expected, it is believed that it has made some contributions to the chemistry of heloderma venom. Many of the observations of Santesson have been confirmed. It has been shown that the toxic principle is very readily adsorbed by all kinds of precipitates.* The observation of Santesson that the venom contains a good deal of a protein precipitable by acetic acid has been confirmed. This precipitate, which Santesson classed with the nucleins, but which may be a mucin, is toxic. Santesson believed this "nuclein"

*Cf. the chapter on adsorption for a more detailed account.

itself to be a part of the toxic principle. In view of the great ease with which the latter is adsorbed it is not likely that this is the case. If it were it would be necessary to assume that the venom contained two different active principles with similar action. That two principles are present, the "nuclein" and an albumose, is actually assumed by Santesson. It was possible to show that the "albumose" is not such, since very active solutions were obtained which were quite free from substances giving the biuret reaction. Unfortunately through lack of material it was not possible to obtain the active principle in sufficient quantity for chemical study or analysis; but methods have been described by which with larger quantities of material this ought to be possible.

The venom was also studied in another direction. It was examined for some of the commoner enzymes. For this purpose 1 per cent aqueous solution of the dried venom was prepared, and used unfiltered because some enzymes are not soluble in water. This solution with suspended particles in it was used for all the experiments except those on lipase.

Diastase.

The following mixtures were placed in small test tubes:

No. 1. 0.25 c.c. venom suspension + 0.25 c.c. 1 per cent boiled starch solution, 1 drop toluol.
No. 2. The same.
No. 3. The same.

Tubes Nos. 1 and 2 were placed in the thermostat at 37° C. and were stoppered to prevent the evaporation of the toluol. Tube No. 3 remained at room temperature. At intervals a drop was removed from each tube with a clean, dry glass rod, placed on a white porcelain plate, and tested for starch with Lugol's solution diluted fifty times. The following results were obtained:

	2 hours.	4 hours.	12 hours.	24 hours.
No. 1.....	Dark blue.	Dark blue.	Purple.	Weak red.
No. 2.....	Dark blue.	Dark blue.	Purple.	Weak red.
No. 3.....	Dark blue.	Dark blue.	Purple.	Strong red.

It may therefore be concluded that the dried venom has weak diastatic action.

Peptic Ferment.

For the detection of peptic digestion the edestin method of Fuld and Levison* was used. A solution of edestin was prepared of 1 part in 1,000 of N/30 hydrochloric acid. The following tubes were prepared:

No. 1. 0.5 c.c. of venom suspension, 0.5 c.c. edestin solution, 0.1 c.c. toluol.
No. 2. The same.
No. 3. The same.

All three tubes were stoppered to prevent escape of toluol and placed in the thermostat at 37° C. At the end of 4 hours all were filtered and a little sodium chloride in substance added. No trace of a precipitate could be seen. The venom therefore contains a peptic ferment.

*Cf. E. Abderhalden. Handbuch der Biochemischen Arbeitsmethoden, Bd. III, S. 18.

Tryptic Treatment.

To test the venom solution for tryptic enzyme it is only necessary to give it the requisite alkalinity and then let it digest itself, since the venom itself contains a mucine-like substance which is precipitable on acidifying with acetic acid. The following experiments were performed:

No. 1. 0.5 c.c. venom suspension, 0.5 c.c. 0.4 per cent sodium carbonate solution, 0.1 c.c. toluol.
No. 2. The same.
No. 3. The same.

All the tubes were stoppered and set in the thermostat. After 4 hours they were filtered and 4 per cent acetic acid carefully added. A good precipitate formed. A second set of similar tubes was prepared and digested 12 hours. On addition of acetic acid to these a precipitate also formed.

The original 1 per cent venom solution was then treated with just enough weak acetic acid to precipitate the mucine-like substance. This was filtered off. The filtrate was carefully neutralized and tested for tryptic enzyme by the casein method of Gross, Fuld, and Michaelis.* To this end a solution of 0.1 gm. casein in 200 c.c. water containing 10 drops of 10 per cent sodium carbonate was prepared. The following tubes were then prepared:

No. 1. 0.5 c.c. venom suspension, 0.5 c.c. casein solution, 0.1 c.c. toluol.
No. 2. The same.
No. 3. The same.

These were incubated in the thermostat for 12 hours. They were then carefully acidified. Casein was precipitated in two of the tubes.

Neither of the two methods may be very reliable. The method by which the acetic-acid precipitable protein of the venom is used as indicator may have been negative because there is too much of the protein present. If only a small amount of it had been digested this would have escaped detection. The casein method is exceedingly unsatisfactory because of the difficulty of avoiding an excess of acidity. Therefore further experiments were tried. Gelatin plates were poured in Petri dishes with 2 per cent gelatin. Small areas of the gelatin surface were covered with the venom suspension rendered alkaline with sodium carbonate. After considerable lengths of time no digestion of these areas could be detected; they were not depressed below the rest of the surface. Exactly similar experiments were tried, using blood-serum coagulated at 56° C. in place of the gelatin. In these dishes there seemed to be a slight solution of the serum surface over the areas covered with venom suspension; the effect was very slight. It is, therefore, taking all these experiments into consideration, exceedingly doubtful whether any tryptic enzyme at all occurs in the venom.

Invertase.

This experiment was performed by Dr. C. S. Hudson. He was unable with the polariscope to detect any effect on the rotation of cane-sugar solution, the medium being 2 per cent acid with acetic acid.

*Cf. Abderhalden, E. *Op. cit.*, S. 19.

Lipase.

Lipase was determined according to the method suggested by Michaelis.* Some fresh lecithin was prepared from egg-yolk. The yolks were dissolved in an equal volume of 10 per cent sodium choloride and the resulting solution extracted with ether. The ethereal extract was concentrated by means of an air current. The oily residue was extracted, till white, with acetone. The undissolved residue was freed from acetone by exposure to air in a thin layer on paper. Of this preparation a 2 per cent emulsion in water was made by shaking. This emulsion was then used as the reagent.

As lipase is exceedingly sensitive, and as its action is usually comparatively limited, it was decided not to use the venom solution, but to use the finely powered venom in substance. The following tubes were therefore made up:

No. 1. 5 c.c. lecithin emulsion, 0.1 gm. powdered venom, 0.2 c.c. toluol.
No. 2. The same.
No. 3. The same.

Tube No. 3 was examined at once. 5 c.c. of absolute alcohol were added and the mixture filtered. The tube and the filter were then washed with two successive portions of 5 c.c. of absolute alcohol. Tube No. 1 was treated in the same fashion after 6 hours' incubation and No. 2 after 12 hours' incubation. The alcoholic filtrates were all titrated with N/10 potassium hydrate and phenolphthalein as indicator. The following results were obtained:

No. 1. 1.35 c.c. N/10 alkali required.
No. 2. 1.85 c.c. N/10 alkali required.
No. 3. (control). 0.70 c.c. N/10 alkali required.

Subtracting the amount of acidity originally present in the control we obtain:

No. 1. 0.65 c.c. N/10 acid formed.
No. 2. 1.15 c.c. N/10 acid formed.

To make sure of these results a further control was made, bringing the solution to a boil before incubating. In this experiment 0.8 c.c. N/10 acid, very little more than the control (No. 3), was required. It seems, therefore, reasonably certain that the venom contains some lipase.

The occurrence of lipase in heloderma venom is a matter of considerable interest. The occurrence of lipase in various venoms has been reported by Neuberg and Reicher,† and Neuberg and Rosenberg,‡ and was by them and by Noguchi§ brought into relation with hemolysis. Previously Delezenne¶ and Friedemann|| had discovered that neutralized pancreatic juice is hemolytic.

*Cf. Abderhalden, E. *Op. cit.*, S. 22-23.

†Neuberg, C., and Reicher, C. Lipolyse, Agglutination und Hämolyse, Biochemische Zeitschrift, Band 4, S. 281.

‡Neuberg, C., and Rosenberg, E. Lipolyse, Agglutination und Hämolyse, Berliner klinische Wochenschrift, Band 44, S. 54.

§Noguchi, H. On certain thermostabile venom activators, The Journal of Experimental Medicine, vol. 8, p. 87.
Noguchi, H. Ueber einer lipolytische forme der Hämolyse. Biochemische Zeitschrift, Band 6, S. 184.

¶Delezenne, C. Comp. rend. d. l. Soc. Biol. d. Par., T. 55, p. 171 (1903).

||U. Friedemann. Deutsche Med. Woch. 1907.

Indeed, Noguchi showed that pancreas lipase when freed from fat loses its hemolytic power, while the venoms of various snakes are activated by lecithin, triolein, and oleinic acid. Since the venom of *Heloderma* is hemolytic the occurrence of lipase becomes interesting, especially since it has been shown that the venom contains appreciable quantities of alcohol and ether-soluble material. It seemed, therefore, worth while to study the lipolytic action of the venom in detail. Two points seemed important. In the experiments reported above, relatively large amounts of venom were used. It was therefore necessary to determine whether positive results could be obtained with smaller amounts. Furthermore, the thermolability of the venom seemed to demand further investigation, since this point has not been much investigated by previous authors. At the same time the effect of the reaction of the medium, the action on oil instead of lecithin, the effect of manganese, and of bile salts were also considered.

The following series of experiments was set up and all except some of the controls (Nos. 1, 2, 6) incubated for 60 hours. Then 25 c.c. of alcohol and a little phenolphthalein were added to each and the acidity titrated. The controls, Nos. 1 and 2, were titrated at once. From the value obtained the acidity of the control is subtracted and the result represents the acid formed in the given experiment.

No. 1. 1 c.c. 1 per cent venom solution, 10 c.c. 2 per cent lecithin emulsion, 1 c.c. water, 3 drops toluol. This served as the control and was titrated immediately. Cubic centimeters N/10 alkali required for neutralization, 1.5.

No. 2. The same as No. 1. Number of cubic centimeters of N/10 alkali required for neutralization, 1.8.

No. 3. The same as No. 1. The titration, however, was done after 60 hours of incubation at 38° C. and required 4.9 c.c. N/10 potassium hydroxid. Subtracting the average of the controls (Nos. 1 and 2) this leaves 3.25 c.c.

No. 4. 1 c.c. 1 per cent venom solution was heated to 60° C. for 30 minutes. It was cooled; and 10 c.c. 2 per cent lecithin emulsion, 1 c.c. water, and 3 drops of toluol added. The increase in acidity after incubation corresponded to 2.5 c.c. N/10 potassium hydroxid. Evidently the lipase was not completely destroyed by the heating, but only weakened.

No. 5. The same as No. 4. Acidity = 2.1 c.c. N/10 potassium hydroxid.

No. 6. 1 c.c. 1 per cent venom solution was heated to 100° C. for 10 minutes. In all other respects the conditions were as in Nos. 4 and 5. Acidity = 1 c.c. N/10 potassium hydroxid. Evidently the heating had almost completely destroyed the activity.

No. 7. 1 c.c. 1 per cent venom solution, 10 c.c. 2 per cent lecithin emulsion, 0.5 c.c. of 0.2 per cent sodium taurocholate solution, 0.5 c.c. water. Increased acidity after usual incubation 2.7 c.c. N/10 potassium hydroxid. Evidently the bile salt had not increased the lipolysis.

No. 8. 1 c.c. 1 per cent venom solution, 10 c.c. 2 per cent lecithin emulsion, 1 c.c. of an 0.8 per cent solution of manganese sulphate. Increased acidity = 2.8 c.c. N/10 potassium hydroxid. Evidently the manganese salts did not increase the lipolysis.

No. 9. 2 c.c. water, 10 c.c. olive oil which had been rendered neutral by standing in contact with powdered calcium carbonate for some time, 3 drops toluol. Acidity after incubation = 1.5 c.c. N/10 potassium hydroxid.

No. 10. 1 c.c. 1 per cent venom solution, 10 c.c. olive oil, 0.5 c.c. of 0.8 per cent magnesium sulphate solution, 0.5 c.c. water, 3 drops toluol. Increased acidity above No. 9 after incubation = 1 c.c. N/10 potassium hydroxid.

No. 11. 1 c.c. 1 per cent venom solution, 10 c.c. olive oil, 0.5 c.c. of 0.2 sodium taurocholate solution, 0.5 c.c. water, 3 drops toluol. Increased acidity above No. 9 after incubation = 0.7 c.c. N/10 potassium hydroxid. Evidently the lipolysis is far less for olive oil than for lecithin.

No. 12. 1 c.c. water, 10 c.c. 2 per cent lecithin emulsion, 1 c.c. N/10 hydrochloric acid, three drops toluol. After incubation acidity = 13.5 c.c.

No. 13. 1 c.c. 1 per cent venom solution, 10 c.c. 2 per cent lecithin emulsion, 1 c.c. N/10 hydrochloric acid, 3 drops toluol. The acidity after incubation was the same as No. 12.

Evidently lecithin is very easily hydrolyzed by mineral acid, and this hydrolysis is not influenced by the venom. It may be that the ease with which lecithin is hydrolyzed by acid accounts for some of the results obtained in the course of this investigation and by other investigators. It may be one of the factors upon which the greater lipolysis of lecithin depends, for the small quantities of acids liberated at the beginning by enzyme action probably tend even without the further help of any enzyme to hydrolyze the lecithin further.

No. 14. 1 c.c. water, 10 c.c. 2 per cent lecithin emulsion, 1 c.c. N/10 potassium hydroxid, 3 drops toluol. After incubation acidity = 1.9 c.c. N/10 potassium hydroxid.

No. 15. 1 c.c. 1 per cent venom solution, 10 c.c. 2 per cent lecithin emulsion, 1 c.c. N/10 potassium hydroxid, 3 drops toluol. After incubation, acidity after subtracting the control No. 14 = 1.6 c.c. N/10 potassium hydroxid.

Evidently, while the venom lipolyzes in a solution of this alkalinity, it is more active in less alkaline ones.

Since these experiments demonstrated that too great an acidity had an action independent of the enzyme, and since too great alkalinity diminished lipolysis, a further series of experiments was undertaken under conditions which should insure continued neutrality in the solution. To attain this end, a solution of 9 gm. disodic phosphate (Na_2HPO_4) and 1 gm. of monosodic phosphate (NaH_2PO_4) in 100 c.c. of water was employed, as recommended by L. J. Henderson. At the same time the destructive effect of high temperatures was confirmed. The following experiments were set up:

No. 16. 1 c.c. 1 per cent venom solution, 10 c.c. 2 per cent lecithin emulsion, 1 c.c. water, 3 drops toluol. This was titrated at once. Acidity = 2.4 c.c.

No. 17. 1 c.c. 1 per cent venom solution, 10 c.c. 2 per cent lecithin emulsion, 1 c.c. water, 3 drops toluol. Acidity after incubating and subtracting control No. 16 = 1.2 c.c. It is not clear why in this experiment a lower value was obtained than in No. 3.

No. 18. 1 c.c. 1 per cent venom solution, 10 c.c. 2 per cent lecithin emulsion, 2 drops toluol, 1 c.c. phosphate solution. In order that the phosphate might not interfere with the titration, this solution was exhausted at once with ether, the ether evaporated with an air current, the residue dissolved in alcohol and titrated. Acidity = 2.1 c.c. N/10 potassium hydroxid.

No. 19. This experiment was in every way identical with No. 18, except that the mixture was incubated before extraction and titration. Acidity = 3.7 c.c. N/10 potassium hydroxid or, subtracting control (No. 18), 1.6 c.c.

No. 20. The same as No. 19, except that it remained in the incubator 12 hours longer. Acidity = 4.7 c.c. N/10 potassium hydroxid or, subtracting control (No. 18), 2.6 c.c. Evidently keeping the medium neutral did not have a very great effect.

No. 21. 1 c.c. 1 per cent venom solution heated to 100° C. for 30 minutes, 10 c.c. 1 per cent lecithin emulsion, 1 c.c. water, 3 drops toluol. After incubation and subtraction of control (No. 16) acidity = 0.2 c.c. N/10 potassium hydroxid. Evidently boiling, as also appears from the first series of experiments, destroys the lipolytic power.

Dry Venom Heated to 60°.

All the experiments were performed exactly as in the first series, so that they need not be redescribed. The following results were obtained:

Diastase: Absent.
Pepsin: Present.
Trypsin: Probably absent.
Lipase: After 6 hours' digestion, subtracting control, 0.3 c.c. N/10 alkali required. After 12 hours' digestion, 0.8 c.c. N/10 alkali.

Dried Blood of Heloderma, 1910.

Diastase: Doubtful.
Pepsin: Absent.
Trypsin: Slight. (Casein method only.)
Lipase: After 6 hours, subtracting control, 0.3 c.c. N/10 alkali. After 12 hours, 0.5 c.c. N/10 alkali.

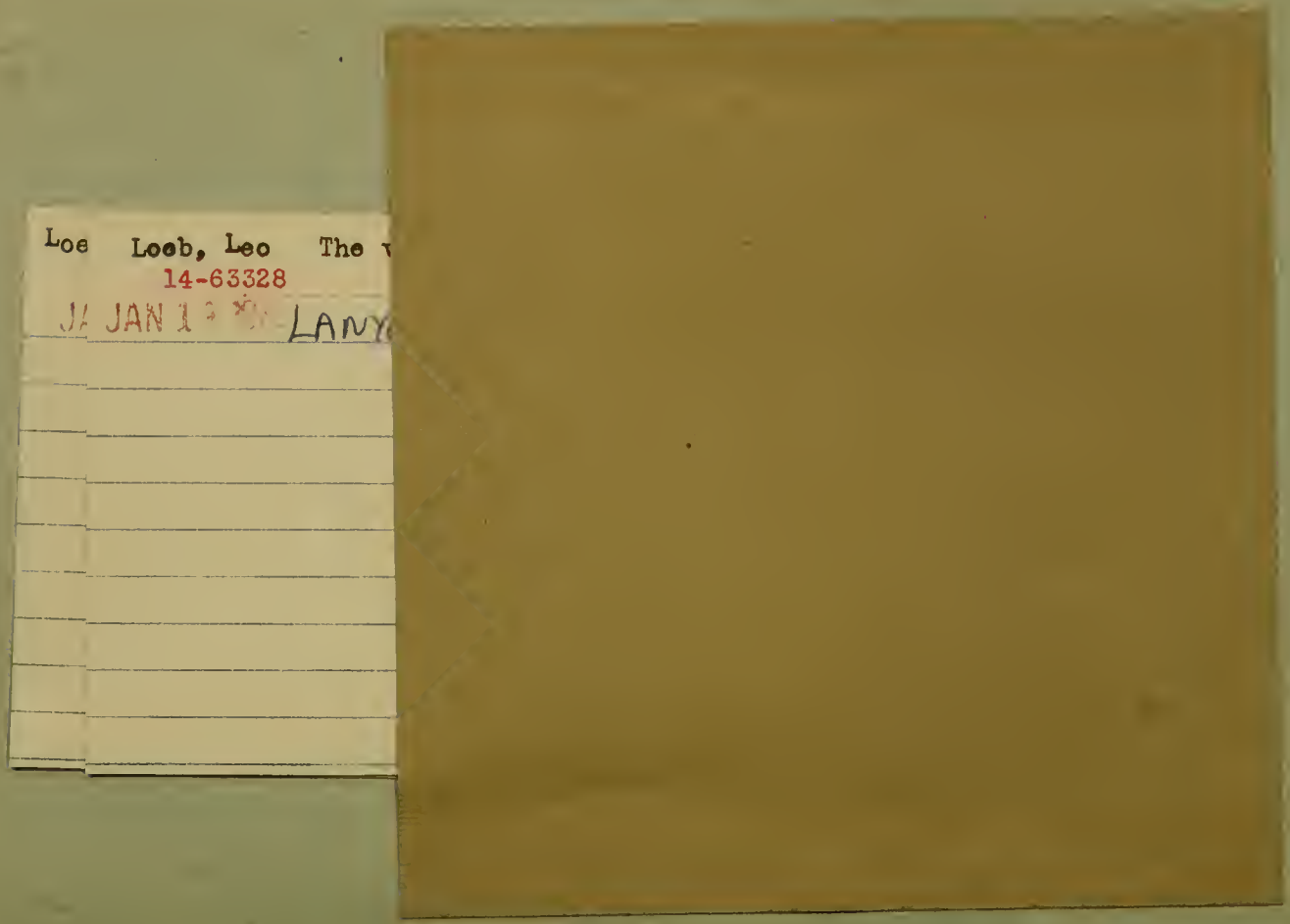
Dried Blood of Heloderma (Second Specimen).

Diastase: Weak.
Pepsin: Absent.
Trypsin: Present, slight. (Casein method only.)
Lipase: After 6 hours 0.2 c.c. N/10 alkali required. After 12 hours 0.6 c.c. N/10 alkali required.

The Venom Gland (Dried and Powdered).

Diastase: Absent.
Pepsin: Trace.
Trypsin: Doubtful trace, probably absent. (Casein method only.)
Lipase: After 6 hours' incubation required 0.6 c.c. N/10 alkali. After 12 hours' incubation required 1.1 c.c. N/10 alkali.

The absence of even a trace of diastase in the dried gland is interesting. The diastase present in the venom must be supplied by some one of the other glands pouring their secretion into the mouth.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